37èmes Journées Nationales de Biologie Clinique
2èmes Journées du Syndicat Tunisien des Biologistes Privés

Poster 328

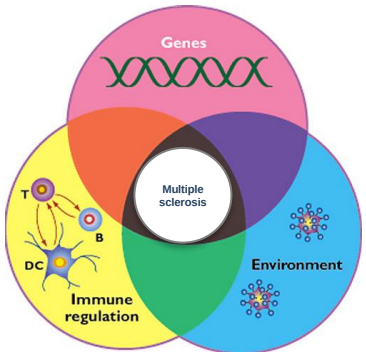
Vitamin D metabolism and immune response in Multiple sclerosis: A serological, transcriptomic and functional study

Sawsan FEKI[a], Y BEN ALI[a], M NAIFAR[b], R FAKHFAKH[a], S SAKKA[c], O ABIDA[a], H HACHICHA[a], F AYEDI[b], M DAMMAK[c], C MHIRI[c], H MASMOUDI[a]

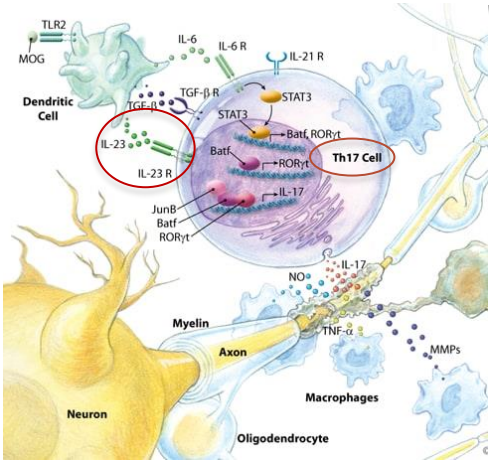
a-Laboratory of Immunology; b-Laboratory of Biochemistry, Habib Bourguiba University Hospital, University of Sfax, Tunisia; c-Department of Neurology, Habib Bourguiba University Hospital, Faculty of Medicine, University of Sfax, Tunisia

INTRODUCTION

A combination of genetic, environmental, and immunologic factors contributes to the development of Multiple sclerosis (MS) disease

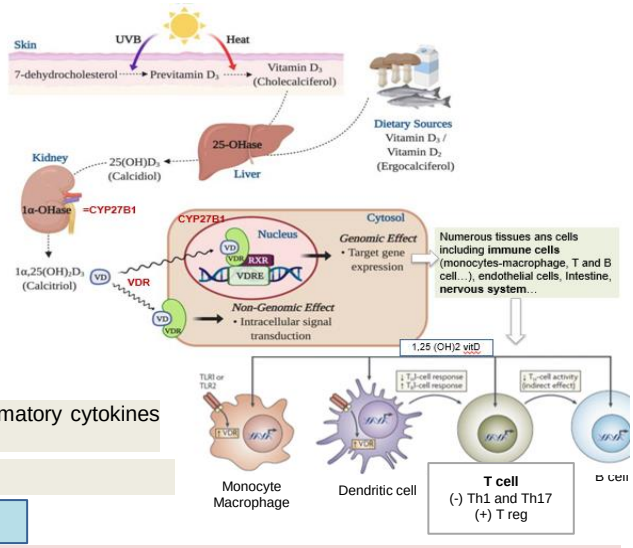


→ The **IL23/Th17 pathway** seems to play an important role in the pathogenesis of MS.



→ The immunomodulatory role of **vitamin D** (Vit D) and its possible involvement in the susceptibility to MS have been well documented.

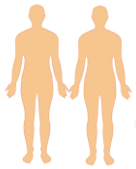
→ The vitamin D receptor (**VDR**) and the activating enzyme (**CYP27B1**) have a key role in Vit D metabolism and its biologic effects.



AIM: To study the effects of VitD on the immune system during MS: VitD status with the inflammatory cytokines (innate immunity) and VitD-related genes with the Th17 pathway (adaptive immunity).

Material and methods

A case-control study including a Tunisian population (n=318) ➔ 108 MS patients ------ 210 healthy controls (HC)



- Serum➔ 108 MS patients /210 HC
- mRNA ➔ 45 MS patients/45 HC
- PBMC ➔ 5 MS patients / 6 HC

Serological study: Serum levels of **25 (OH) D** (circulating form of VitD) and **IL17A** determined using ELISA
Serum levels of **IL-6, IL-8, IL-10, TNF-a, IL12p70, IL-1b** by Cytometer Bead Array(CBA) Technology

Transcriptional study: *VDR, CYP27B1* and *IL23R* gene expression in PBMC (peripheral blood mononuclear cells) using RT-PCR.

Cell culture step of PBMC with/without VitD stimulation for MS patients and healthy controls.
→The production of IL17A was analyzed in the supernatant of culture.

	MS patients (n=45)	HC (n=45)
Mean age +/- SD	32.8 ± 10.1 ans	31.35 ± 7.6 ans
Sex-ratio	0.406	0.653
EDSS +/- SD	1.7±1	---
IgG Index	1 ± 0.57	---
OCB detection in CSF	OCB (+) (85.72 %) OCB (-) (14.28 %)	---

Gene	Location	Transcripts	Protein
VDR	Chromosome12 : position 12q13.11	· NM_000376.3 · NM_001017535.2 · NM_001017536.2 · NM_001364085.2 · NM_001374661.1 · NM_001374662.1	Receptor of vitamin D (VDR)
CYP27B1	Chromosome12 : position 12q14.1	• NM_000785.4	Enzyme 1α-hydroxylase (CYP27B1)
IL23R	Chromosome1 : position 1p31.3	• NM_144701.3	Receptor of the cytokine IL23 (IL23R)

Results

Figure1: Comparative distribution of 25 (OH) Vitamin D status in MS patients and healthy controls

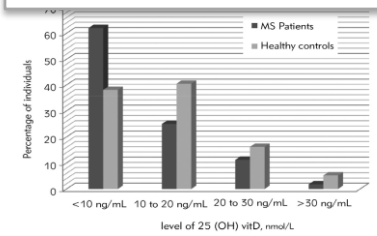


Figure2 : Comparative mean values of circulating inflammatory cytokines in oligoclonal bands (OCB)-positive Versus OCB negative MS patients

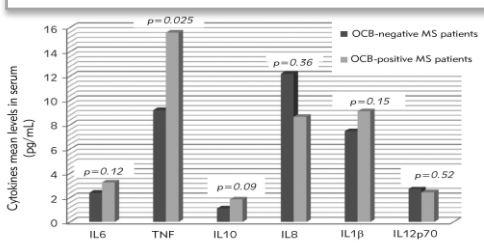
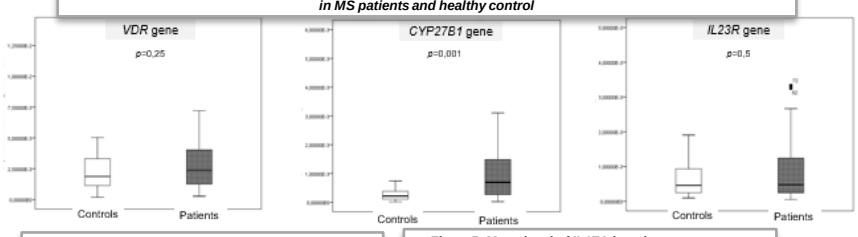


Table 1: Level of circulating IL17 in patients and controls

	Mean level of circulating IL17A (SEM) (pg/ml)		p value
Disease status	MS group (n=45)	Controls (n=45)	0.054
	3.01 (0.60)	2.74 (0.11)	

Figure3: Comparative Box plots showing the distribution of the relative expression of VDR, CYP27B1 and IL23R in MS patients and healthy control



Tables 2 and 3: Correlation analysis of the studied parameters

Genes relative expression in the population (n=90)		VDR	CYP27B1	IL23R
VDR	Coefficient of correlation	----	0.418	0.238
	p value		**<0.001**	**0.026**
CYP27B1	Coefficient of correlation	0.418	----	0.321
	p value	**<0.001**		**0.002**
IL23R	Coefficient of correlation	0.238	0.321	----
	p value	**0.026**	**0.002**	

Genes relative expression in MS patients (n=45)		VDR	CYP27B1	IL23R
VDR	Coefficient of correlation	----	0.731	0.471
	p value		**<0.001**	**0.018**
CYP27B1	Coefficient of correlation	0.731	----	0.514
	p value	**<0.001**		**0.009**
IL23R	Coefficient of correlation	0.471	0.514	----
	p value	**0.018**	**0.009**	
EDSS	Coefficient of correlation	0.543	-0.505	0.385
	p value	**0.02**	**0.032**	0.115

Figure4: Mean level of IL17A in culture supernatant (pg/ml) in the total population

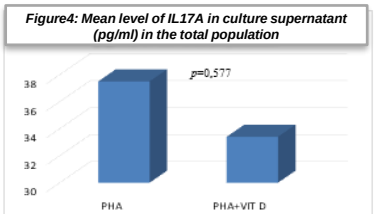
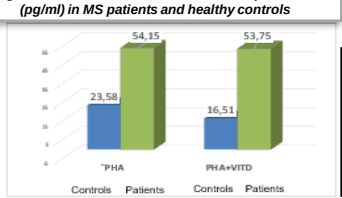


Figure5: Mean level of IL17A in culture supernatant (pg/ml) in MS patients and healthy controls



Conclusion

In conclusion, our study confirmed the reported association of VitD deficiency with MS in our Tunisian population.

The expression of the genes involved in the metabolism of this vitamin (*VDR and CYP27B1*) seems to be inter-correlated and in relation with the IL23/Th17 pathway.

The stimulation with VitD tends to inhibit differentiation towards the Th17 pathway.