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Leukocyte adhesion molecule dynamics after Natalizumab withdrawal in Multiple Sclerosis

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ABSTRACT

Cell-adhesion molecules (CAMs) dynamics in Multiple Sclerosis (MS) patients have been widely studied after Natalizumab (NTZ) introduction. However, their temporal dynamics after NTZ withdrawal (NTZ-W) has not been described. We prospectively evaluate changes in the expression levels of CAMs (CD49d, CD29, L-Selectin and CD11a) involved in T cell migration of 22 MS patients after NTZ-W. CD49d, CD29 and CD11a expression experienced a continuous increase expression two months after NTZ-W and Cd49d expression at month six after NTZ-W correlated to NTZ treatment duration, both in $CD45^+CD4^+$ and $CD45^+CD8^+$. CD49d expression up to month three after NTZ-W was related to MS activity in $CD45^+CD8^+$ at the end of the study. Results from this study suggest that patients with a longer NTZ treatment are more susceptible to present a "molecular rebound" after NTZ-W. CD49d determination may be a useful tool to closely monitor MS activity in patients who interrupt NTZ.

1. Introduction

Natalizumab (NTZ) (Tysabri® Biogen Idec) is a humanized IgG4/kappa monoclonal antibody considered as one of the most effective treatments in Multiple Sclerosis (MS) [1]. NTZ recognizes the α subunit of the α4β1 integrins (CD49d/CD29, also called Very Late Antigen 4, [VLA-4]) located on the surface of leukocytes, blocking the interaction between both molecules to the endothelial receptor vascular-cell adhesion molecule 1 (VCAM-1) and the mucosal adhesion-cell adhesion molecule 1 (MADCAM-1). In this way, NTZ impairs the passage of leukocytes across the blood brain barrier (BBB) and decreases inflammation in the central nervous system (CNS) [2].

The major risk of NTZ treatment is John Cunningham virus (JCV)-mediated progressive multifocal leukoencephalopathy (PML). PML risk in MS patients under NTZ can be stratified according to the anti-JCV antibody status, prior use of immunosuppressants and duration of NTZ treatment [3]. Nonetheless, given the lack of precise individual PML prediction of this stratification, new markers have been proposed. While the quantification of anti-JCV antibody (antibody index) is the most valuable candidate [4], other T cell adhesion molecules (CAMs) involved in cell arrest and migration toward inflammation sites such as (CD11a/CD18, also called lymphocyte function associated antigen-1 [LFA-1]) and L-Selectin (CD62L) are, currently, under discussion [5–8]. Owing to the PML risk, NTZ withdrawal (NTZ-W) and subsequent switching to other therapies after a two-month washout period must be considered in clinical practice [9]. Although some studies suggest an absence of MS activity exacerbation after NTZ- W [10,11], MS cases have been reported with a severe clinical or radiological activity occurring shortly after NTZ cessation and Fingolimod initiation [12,13]. Concerns about MS rebound leads to the need to identify this subgroup of patients in order to closely monitor them and thus, prevent MS activity after NTZ-W.

Despite CAMs kinetics after NTZ initiation have being widely described, studies focusing on these molecules after NTZ-W are scarce. Since CAMs play a central role in MS pathophysiology and, specifically in MS patients treated with NTZ, we aimed to evaluate the changes in the expression levels of cell trafficking CAMs involved in T cells migration at short term after NTZ-W. We further addressed whether duration of NTZ treatment or MS activity during NTZ treatment may have an influence over CAMs dynamics. Finally, we investigated CAMs dynamics as a potential tool to predict MS disease in patients with interrupted NTZ treatment.

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2. Subjects and methods

2.1. Subjects

Twenty-two MS patients who fulfilled the revised McDonald Criteria were enrolled in this observational and prospective study at the time of NTZ-W [14]. All patients began intravenous (i.v) treatment with NTZ (300 mg monthly) at the MS Unit of Bellvitge University Hospital. Baseline characteristics of NTZ-W and treatment-naïve patients are detailed in Table 1.

The study (n° PR 091/13) was approved by the local ethics committee and all participants gave written informed consent.

All patients were a high risk of developing PML by testing positive for anti-JCV antibody and being under NTZ treatment for at least two years [3]. Exclusion criteria consisted of the presence of relapses, infections or receiving corticoids within a six month period before study onset.

According to the washout protocol for MS in our Unit with interrupted NTZ, patients received 1 g of i.v methylprednisolone (MTP) at month one, two and three after NTZ-W. Fingolimod was started at month two after NTZ-W and further administrated daily.

Peripheral blood (PB) samples were collected at months one, two, three and six (M1, M2, M3 and M6) after NTZ-W and prior to MTP.

The control group consisted of nine treatment-naïve MS patients who had not received corticoid treatment and had not experienced relapses within six month previous to their inclusion in the study (Table 1). Two PB samples were collected six months apart. The first sample was used to perform CAMs kinetics comparisons with NTZ-treated patients.

2.2. Clinical and radiological data

Patients were clinically evaluated every month within the first three months and at months 6 and 12 after NTZ-W. The Expanded Disability Status Scale (EDSS) was evaluated at the time of NTZ initiation and NTZ final infusion as well as at every clinical evaluation of the study. Relapse was defined as new or worsening neurological symptoms sustained for at least 24 h in the absence of fever or infection. The annualized relapse rate (ARR) was recorded before and during NTZ treatment and was assessed for the period of the study.

A brain magnetic resonance imaging (MRI) was performed on all patients within one month previous to the enrollment and at month 6 and 12 after NTZ-W. All studies were performed at the same centre (Institut de Diagnòstic per la Imatge, *IDI*) using a 1.5 Tesla system, and included axial/sagittal T2-weighted images (WI) and T1- WI with and without gadolinium. The number of T2-WI lesions and gadolinium enhancing lesions was identified in a blind manner by one neuroradiologist (CM).

We further described the CAMs kinetics during the first six months (short term) and the clinical and radiological features up to 12 months after NTZ-W.

Table 1
Baseline characteristics of NTZ withdrawal and treatment-naïve patients.

Epidemiological characteristics	NTZ-W n = 22	Treatment-naïve, n = 9	p-Value
Age at MS diagnosis, median (range), y	25.3 (15.5–52.0)	30.2 (18.4–43.4)	0.317
Age at NTZ initiation, median (range), y	37.9 (24.8–60.2)	–	–
Age at NTZ- W, median (range), y	43.4 (28.3–63.3)	–	
Female, n (%)	15 (68.2)	7 (77.8)	0.689
NTZ treatment duration, median (range), y	5.5 (2.6–7.9)	–	–
MS Follow- up, median (range), y	16.5 (8.2–33.0)	15.9 (9.7–36.5)	0.663
JCV-index >1.5, n (%)	22 (100)	–	–
Previous immunosuppressants, n (%)	0	0	–
Clinical characteristics			
ARR before NTZ, mean (SD)	1.01 (0.67)	0.18 (0.09)[a]	–
ARR during NTZ, mean (SD)	0.37 (0.67)	–	
EDSS at NTZ onset, median (IQR)	3.0 (2.5–4.5)	1 (0–2.5)[b]	–
EDSS at NTZ-W, median (IQR)	3.0 (2.5–4.0)	–	
Radiological characteristics			
T2 lesions at NTZ-W, n (%)		–	
<20	6 (27.3)		
20–40	10 (45.5)		
>40	6 (27.3)		
Gad lesions at NTZ-W	0 (0)	–	

MS: multiple sclerosis; NTZ-W: natalizumab withdrawal; JCV: John Cunningham virus; ARR: annualized relapse rate; EDSS: Expanded Disability Status Scale; Gad: gadolinium; y: years; SD: standard deviation; IQR: interquartile range.
[a] ARR in treatment-naïve MS patients at first sample.
[b] EDSS in treatment-naïve MS patients at first sample.

2.3. Venous blood sample preparation

PB fresh samples from MS patients were collected in 10 ml EDTA tubes (BD Vacutainer®). Samples were processed within no more than six hours from collection. Samples were incubated with fluorochrome-conjugated monoclonal antibodies (mAb) and isotype-matched negative controls for 30 min at room temperature (Supplementary Table 1). Red blood cells were lysed with 1 ml ACK Lysing Buffer 1× (Lonza) two times for10 min in darkness. After two washes with 1× PBS 2 mM EDTA 0.5% BSA, cells were fixed with 0.1% of paraformaldehyde and kept at 4 °C in darkness for <12 h before cytometry analysis.

2.4. Flow cytometry

The expression levels of CAMs were investigated in two T populations; $CD45^+CD4^+$ and $CD45^+CD8^+$. As we were interested in the unblocked CD49d, we used a detection antibody of the same epitope specificity as NTZ. For the rest of CAMs, the total surface expression was measured [15,16]. A Gallios Beckman Coulter Flow Cytometer was used for acquisition fluorescence data of CAMs on peripheral blood mononuclear cells (PBMCs). Our acquisitions were a minimum of 250.000 cells in each sample. Subsequent CAMs expression analysis was performed with FCS Express 5 Plus (DeNovo Software).

Relative fluorescence intensities (rfi) were calculated from the geometrical (geo) mean fluorescence intensity (mfi) in relation to the total $CD45^+$ population and the auto-fluorescence of the cells; according to the following formula: geo mfi (CAM to population of interest) − geo mfi (isotype control IgG1 to population of interest) / geo mfi ($CD45^+$ population) − geo mfi (IgG1 of $CD45^+$ population) ∗ 1000.

2.5. Statistical analyses

The non-parametric paired Wilcoxon matched signed-rank was used to compare the CAMs rfi expression between M1 and the subsequent time points (M2, M3 and M6) in NTZ-treated patients, as well as the paired match samples at M1 and M6 in the treatment-naïve patients. We used the Wilcoxon rank-sum test to compare the CAMs rfi expression between treatment-naïve and NTZ-treated patients at multiple time-points. The strength of associations between clinical or radiological variables and rfi levels of CAMs was analyzed using the Spearman rank correlation coefficient. All statistical analyses were adjusted by Bonferroni and $p < 0.05$ was considered statistically significant. All statistical analyses were performed using STATA (64-bit) software and graphs were created using GraphPad Prism software.