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Potential role of IL-13 in neuroprotection and cortical excitability regulation in multiple sclerosis

S Rossi[1,2*], R Mancino[3*], A Bergami[4], F Mori[1,2], M Castelli[1,2], V De Chiara[1,2], V Studer[1,2], G Mataluni[1,2], G Sancesario[5,6], V Parisi[7], H Kusayanagi[1,2], G Bernardi[1,2], C Nucci[3], S Bernardini[5,6], G Martino[4], R Furlan[4§] and D Centonze[1,2§]

Abstract

Background: Inflammation triggers secondary neurodegeneration in multiple sclerosis (MS).

Objectives: It is unclear whether classical anti-inflammatory cytokines have the potential to interfere with synaptic transmission and neuronal survival in MS.

Methods: Correlation analyses between cerebrospinal fluid (CSF) contents of anti-inflammatory cytokines and molecular, imaging, clinical, and neurophysiological measures of neuronal alterations were performed.

Results: Our data suggest that interleukin-13 (IL-13) plays a neuroprotective role in MS brains. We found, in fact, that the levels of IL-13 in the CSF of MS patients were correlated with the contents of amyloid-$\beta_{1\text{-}42}$. Correlations were also found between IL-13 and imaging indexes of axonal and neuronal integrity, such as the retinal nerve fibre layer thickness and the macular volume evaluated by optical coherence tomography. Furthermore, the levels of IL-13 were related to better performance in the low-contrast acuity test and Multiple Sclerosis Functional Composite scoring. Finally, by means of transcranial magnetic stimulation, we have shown that GABAA-mediated cortical inhibition was more pronounced in patients with high IL-13 levels in the CSF, as expected for a neuroprotective, anti-excitotoxic effect.

Conclusions: The present correlation study provides some evidence for the involvement of IL-13 in the modulation of neuronal integrity and synaptic function in patients with MS.

Keywords

amyloid-β, inflammation, neurodegeneration, optical coherence tomography, TMS

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Introduction

Imbalance between pro-inflammatory cytokines released by T helper 1 (TH1) and T helper 17 (TH17) lymphocytes (interleukin-1β, IL-1β; tumour necrosis factor α, TNFα; interferon γ, INFγ; IL-17) and anti-inflammatory cytokines released by T helper 2 (TH2) cells (IL-4, IL-5, IL-10 and IL-13) substantially contributes to brain damage in multiple sclerosis (MS).[1–3] In this disorder, secondary neurodegenerative damage is heavily associated with the activity of pro-inflammatory cytokines,[4] which exert their neurotoxic effects through various mechanisms, including the modulation of synaptic transmission and the promotion of glutamate-mediated excitotoxicity. TNFα, INFγ and IL-1β, in fact, increase in the cerebrospinal fluid (CSF) of patients with MS,[5–7] and have been found to enhance

[1]Clinica Neurologica, Dipartimento di Neuroscienze, Università Tor Vergata, Rome, Italy.
[2]Centro Europeo per la Ricerca sul Cervello (CERC)/Fondazione Santa Lucia, Rome, Italy.
[3]Clinica Oculistica, Dipartimento di Biopatologia, Università Tor Vergata, Rome, Italy.
[4]Neuroimmunology Unit-DIBIT, INspE, Division of Neuroscience, San Raffaele Scientific Institute, Milan, Italy.
[5]Dipartimento di Medicina Interna, Università Tor Vergata, Rome, Italy.
[6]UOC Biologia Molecolare Clinica, Dipartimento di Medicina di Laboratorio, Policlinico Tor Vergata, Rome, Italy.
[7]UOCIRCCS Fondazione G. B. Bietti, Rome, Italy.
[*]SR and RM are equally contributing authors.
[§]RF and DC are equal Senior Authors.

Corresponding author:
Diego Centonze, Clinica Neurologica, Dipartimento di Neuroscienze, Università Tor Vergata, Via Montpellier 1, 00133 Rome, Italy
Email: centonze@uniroma2.it.

excitatory synaptic transmission and excitotoxic damage in vitro.[4,8–10]

The potential role of TH2 cytokines in the control of synaptic transmission and neuronal survival during brain inflammation is, conversely, less explored. In this respect, the effects of IL-5 and IL-13 on synaptic function are still unknown, but IL-4 has been found to mediate neuroprotection by favouring glutamate clearance from astrocytes,[11] and enhancing GABA signalling in neurons.[12] IL-10, on the other hand, significantly attenuates the neurotoxic effects of glutamate,[13] and promotes survival of GABAergic neurons.[14] A neuroprotective role of IL-13 released from microglial cells has also been documented in rats,[15] although IL-13 may also have some pro-inflammatory actions in experimental autoimmune encephalomyelitis (EAE).[16]

The aim of the present investigation was to explore the possible role of TH2 cytokines in the neurodegenerative damage associated with MS. From its early phases, MS causes diffuse neuronal damage[17–19] and defective neurotransmission favouring glutamate over GABA transmission.[4,20–25] Here, we tried to correlate the CSF levels of IL-4, IL-5, IL-10 and of IL-13 with amyloid-$\beta_{1\text{-}42}$, a soluble marker of neuronal damage[26,27] recently found to be involved also in pathological MS processes.[28,29] A similar correlation analysis was performed between TH2 cytokines and retinal nerve fibre layer (RNFL) thickness evaluated with optical coherence tomography (OCT), since this imaging parameter has been convincingly associated with the neurodegenerative damage of MS.[30–33] Finally, to provide insights into the potential mechanisms of TH2 cytokine-induced neuroprotection in MS, we also evaluated the correlation between anti-inflammatory cytokine levels and measures of GABA- and glutamate-mediated cortical inhibition or facilitation by transcranial magnetic stimulation (TMS).

Materials and methods

The study was approved by the Ethics Committee of the University Hospital Tor Vergata, Rome.

MS patients

We collected CSF from 52 patients (37 females and 15 males, aged 23–56 years), admitted to the neurological clinic of the University Hospital Tor Vergata of Rome, and later diagnosed as suffering from relapsing–remitting (RR) MS. After their admittance, patients underwent, in sequence, brain (and in selected cases also spinal) magnetic resonance imaging (MRI) scan, TMS (if agreed), ophthalmologic examination (if applicable), disability assessment (if applicable) and CSF withdrawal within 24 h. Corticosteroids or other MS-specific immunoactive therapies were initiated later when appropriate. In all instances, patients underwent detection of oligoclonal banding in the CSF (present in 90% of the cases).

The diagnosis of MS was established at the end of the diagnostic protocol by clinical, laboratory and MRI parameters, and matched published criteria.[34,35] In total, 29 subjects were studied during exacerbations (relapsing subjects, 20 females and nine males; mean age 33.7 ± 7.5 years) and 23 during remission (remitting subjects, 17 females and six males; mean age 35.3 ± 10.2 years). Exacerbation was defined as the development of new symptoms or worsening of a pre-existing symptom, confirmed by neurological examination, lasting at least 48 h, and occurring after a period of stability of 30 days or more. MS patients who were clinically stable for at least 3 months prior to enrolment, and who did not present Gd-enhancing lesions on MRI, were considered to be in remission. Disease duration was estimated as the number of years from first episode of focal neurological dysfunction indicative of MS.

As controls, we used CSF from 29 age- and gender-matched individuals (20 females and nine males, aged 25–58 years) without inflammatory or degenerative diseases of the central or peripheral nervous system. These subjects underwent lumbar puncture because of a clinical suspicion of acute peripheral neuropathy, meningitis, or subarachnoidal haemorrhage, which were not confirmed. All the subjects gave their written informed consent to the study.

MRI acquisition and analysis

Three Tesla MRI scan consisted of dual-echo proton density, FLAIR, T2-weighted spin-echo images and pre-contrast and post-contrast T1-weighted spin-echo images. All images were acquired in the axial orientation with 3 mm-thick contiguous slices. T2 lesion volume was determined by manual tracing, and the presence of gadolinium-enhancing (Gd+) (0.2 ml/Kg e.v.) lesions was assessed by a neuroradiologist who was unaware of the patients' clinical details.[36]

CSF determination of TH2 cytokines

CSF was centrifuged to eliminate cells and cellular debris and immediately stored at -80°C until analysed using Bio-Plex Multiplex Cytokine Assay (Bio-Rad Laboratories), according to the manufacturer's instructions. Concentrations of IL-4, IL-5, IL-10, and IL-13 (Bio-Rad Catalog # 171-A11127, #171-304000, #171-305000) were calculated according to a standard curve generated for each target and expressed as pg/ml.

CSF determination of amyloid-$\beta_{1\text{-}42}$

Immediately after its collection, CSF was centrifuged and stored at -80°C until analysed.[28,36] Levels of amyloid-$\beta_{1\text{-}42}$ were determined in a subset of MS patients ($n = 30$) and control subjects ($n = 15$), according to standard procedures, using commercially available sandwich enzyme-linked immunosorbent assays (Innotest β-Amyloid 1-42, Innotest h-tau Ag, Innogenetics, Ghent, Belgium). The absorbance of the reaction product was read at 450 nm. The biomarker concentrations in the samples were calculated based on the amyloid-$\beta_{1\text{-}42}$ standard sigmoid curve equation, as reported elsewhere.[28,37]

Clinical assessment of disability

Remitting MS patients underwent neurological examination in order to perform correlation analyses between measures of disability and CSF contents of TH2 cytokines. The Expanded Disability Status Scale (EDSS) score is a 10-point disease severity score derived from nine ratings for individual neurological domains.[38] The Multiple Sclerosis Functional Composite (MSFC) includes a timed 25-foot walk, a timed nine-hole peg test, and the 3-s version of the Paced Auditory Serial Addition Test (PASAT). Each score was standardized against a reference population to create a *Z*-score. The three *Z*-scores were averaged to give an overall standardized score for each patient, which indicates the number of standard deviation units above (better than) or below (worse than) the reference population.

Ophthalmologic assessment

Medical history with respect to visual symptoms was taken from all MS subjects. Self-report and physician report were confirmed by record review.

A subset of remitting MS patients ($n = 18$) without history of optic neuritis and ophthalmological disease underwent measurement of RNFL thickness and macular volume (MV) for both eyes using Stratus OCT™ Optical Coherence Tomography (software version 4.0.2, Carl Zeiss Meditec, Inc.). For the study, scanning was performed after pharmacological dilation. Average RNFL thickness for 360° around the optic disc was recorded. Also, temporal quadrant (TQ, 316–45°) thickness, superior quadrant (SQ, 46–135°) thickness, nasal quadrant (NQ, 136–225°) thickness and inferior quadrant (IQ, 226–315°) thickness were measured. Values were adjusted for age. One randomly chosen eye from each subject was included in the study. Low-contrast visual acuity (LCVA) testing was performed for each eye separately using retroilluminated low-contrast Sloan letter charts (1.25% contrast at 2 m). Testing was performed by trained technicians experienced in examination of patients for research studies, and patients wore their habitual glasses or contact lenses for distance correction.

Intracortical circuits of the primary motor cortex

In order to evaluate the potential role of TH2 cytokines in the modulation of cortical excitability, we tested, through a paired-pulse (ppTMS) approach, short interval intracortical inhibition (SICI, believed to be mediated by intrinsic GABAAergic circuits),[39] intracortical facilitation (ICF, believed to follow the preferential recruitment of intrinsic excitatory fibres),[39] short intracortical facilitation (SICF, likely mediated by excitatory cortical interneurons),[40] and long interval intracortical inhibition (LICI, believed to reflect local GABAB-mediated pathways)[41] of the primary motor cortex (M1) of the dominant hemisphere. Hand dominance was defined according to the Edinburgh Handedness Inventory.[42] For these experiments, one figure-of-eight coil, external diameter 70 mm was held tangentially to the scalp over the motor 'hot spot' for the dominant first dorsal interosseus (FDI) muscle. Stimulation intensity for test stimulation (TS) was adjusted in each experiment to evoke a motor evoked potential (MEP) of approximately 1 mV peak-to-peak amplitude in the relaxed FDI.

SICI and ICF were tested using paired TMS with a subthreshold conditioning stimulation (CS) preceding a suprathreshold TS[39,43] at six different interstimulus intervals (ISI) (2, 3, 5, 6, 10 and 15 ms). For SICF, according to an established protocol,[44] six randomly intermixed conditions were presented: TS given alone and TS followed by CS at one of five different ISI (1.5, 2.1, 2.7, 3.7, 4.5 ms). LICI was tested following the protocol adopted by Valls-Solé et al.[41]

For each experiment, 10 responses were collected for the test stimulus alone and for conditioned MEPs at each ISI. Changes in MEP amplitude at each ISI were expressed as percentage of the mean unconditioned MEP amplitude.

Statistical analyses

Differences between groups were analysed by independent samples *t*-tests (two-tailed), independent samples Mann–Whitney test for ordinal data (two-tailed), and the Chi-squared test for categorical data. Correlation analyses were estimated by Pearson test for parametric data and Spearman test for non-parametric data. The significance level was established at $p < 0.05$. All values are reported as mean ± SD.

Results

Effects of acute inflammation on TH2 cytokines in MS

We measured CSF levels of IL-4, IL-5, IL-10 and IL-13 in MS patients ($n = 52$) and in controls ($n = 29$). The description of study subjects is provided in Table 1. The levels of IL-10 were undetectable in almost all subjects enrolled in this study and therefore not further analysed. We found that patients with MS had increased CSF levels of IL-13, both in relapsing and remitting phases ($p < 0.05$ with respect to controls, $p > 0.05$ between subjects in remission or exacerbation), indicating a possible compensatory role of IL-13 in MS, unrelated to acute inflammation. Conversely, IL-4 and IL-5 levels did not vary in comparison with control subjects ($p > 0.05$), but in relation to the presence of acute inflammation among MS patients. In fact, the concentrations of these anti-inflammatory cytokines were lower in relapsing than in remitting subjects, and related to each other ($n = 52$, $r = 0.40$, $p = 0.003$). Statistical significance was, however, reached only in the analyses of IL-5 ($p < 0.05$ between remitting and relapsing subjects). IL-13 concentrations were unrelated with IL-4 ($n = 52$, $r = -0.10$, $p > 0.1$) and IL-5 ($n = 52$, $r = -0.13$, $p > 0.1$) levels in these patients (Figure 1).

The MS patients included in this study were in a relatively early stage of the disease. No correlation was found between the disease duration and the CSF levels of the cytokines analysed (data not shown).

Lack of correlation between TH2 cytokines and brain lesion load

No correlation was found between CSF levels of IL-4 ($n = 52$, $r = 0.03$, $p > 0.1$), IL-5 ($n = 52$, $r = -0.12$, $p > 0.1$), IL-13 ($n = 52$, $r = 0.18$, $p > 0.1$) and T2 brain lesion load in MS subjects (Figure 2).

Correlation between TH2 cytokines and amyloid-β

To see whether IL-4, IL-5, or IL-13 may be markers of the neurodegenerative state in MS brains, we explored the correlation between these cytokines and CSF levels of amyloid-$\beta_{1\text{-}42}$, a recognized indicator of neuronal damage in other neurodegenerative diseases.[26,27]

A strong association was found between IL-13 and amyloid-$\beta_{1\text{-}42}$ CSF levels ($n = 30$, $r = 0.56$, $p = 0.001$). Neither IL-4 ($n = 30$, $r = -0.23$, $p > 0.1$) nor IL-5 ($n = 30$, $r = -0.26$, $p > 0.1$) were correlated with amyloid-$\beta_{1\text{-}42}$ in patients with MS (Figure 3). In agreement with a previous report,[28] amyloid-$\beta_{1\text{-}42}$ levels were significantly reduced in the population of MS subjects, compared with control individuals (MS group: $n = 30$, 249.3 ± 143.9 pg/ml; control group: $n = 15$, 405.3 ± 131.8 pg/ml; $p < 0.05$), suggesting that reduced CSF levels of amyloid-$\beta_{1\text{-}42}$ in MS reflect, as in Alzheimer's disease brains, increased tissue deposition. Furthermore, the CSF levels of amyloid-$\beta_{1\text{-}42}$ were positively related to RNFL thickness, a structural marker of neuronal damage ($n = 16$, $r = 0.6$, $p = 0.005$) (data not shown).

Correlation between TH2 cytokines and neurodegenerative damage

Axonal and neuronal cell loss in MS has been convincingly associated with reduced RNFL thickness and MV at the OCT.[45–47] Thus, to confirm the idea that the TH2 cytokines differentially influence neurodegenerative damage in MS, we investigated the possible relationship between IL-4, IL-5, IL-13 and OCT parameters in patients with MS. Our data showed that neither IL-4 nor IL-5 CSF levels were correlated with degenerative damage at the OCT (Table 2), while IL-13 was significantly and positively associated with better neuronal integrity. In fact, both RNFL thickness and MV were directly correlated with IL-13 CSF levels (Table 2 and Figure 4A and 4B). In line with this, IL-13 levels were also associated with better LCVA ($n = 18$, $r = 0.49$, $p = 0.038$; Figure 4C), an emerging visual functional outcome incorporated successfully into MS clinical trials.[48,49] Conversely, neither IL-4 ($n = 18$, $r = -0.13$, $p > 0.1$) nor IL-5 ($n = 18$, $r = -0.19$, $p > 0.1$) were correlated with LCVA in these subjects. These findings are consistent with an inverse relationship between the levels of IL-13 and the entity of subclinical axonal loss in the central nervous system (CNS) of patients with MS.

Correlation between TH2 cytokines and disability

Neurodegenerative damage has been accounted as the most important factor of sustained disability in MS.[50,51] We have tried to relate the central levels of TH2 cytokines to the extent of disability in MS patients. In line with the described association between IL-13 and neuronal preservation, we found a positively,

Table 1. Characteristics of study population

	Control	MS	*p*
Number	29	52	–
Gender (F/M)	20/9	37/15	>0.1
Age (years)	35.3 ± 7.9	34.5 ± 8.8	>0.1
Disease duration (years)	not applicable	3.6 ± 2.1	–

Values are expressed as mean ± standard deviation.

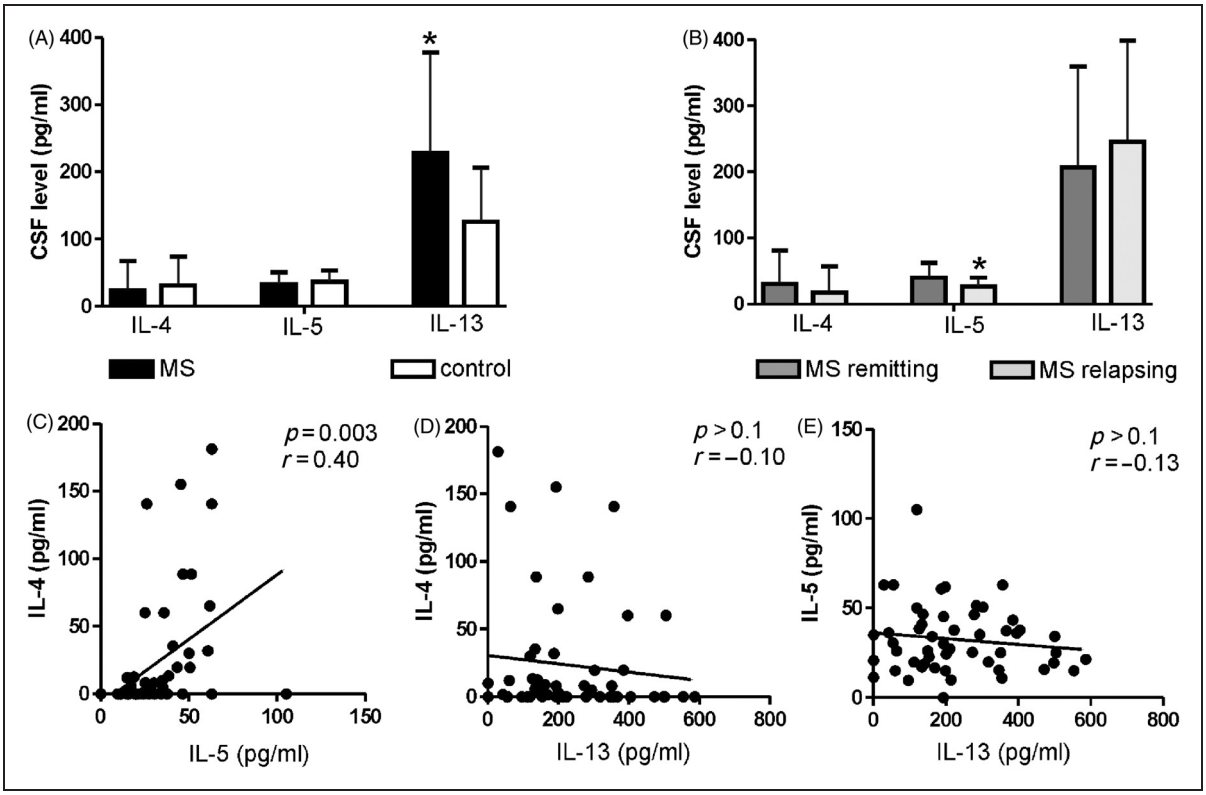

Figure 1. Effects of acute inflammation on TH2 cytokines in multiple sclerosis (MS). A. levels of IL-4 and IL-5 in the cerebrospinal fluid (CSF) of patients with MS were similar to those of controls. IL-13 CSF contents were conversely higher. B. The graph shows that IL-5 levels were lower in relapsing MS patients. C-E. IL-4 and IL-5 CSF levels significantly correlate to each other (C) but not with IL-13 (D, E).* means $p < 0.05$.

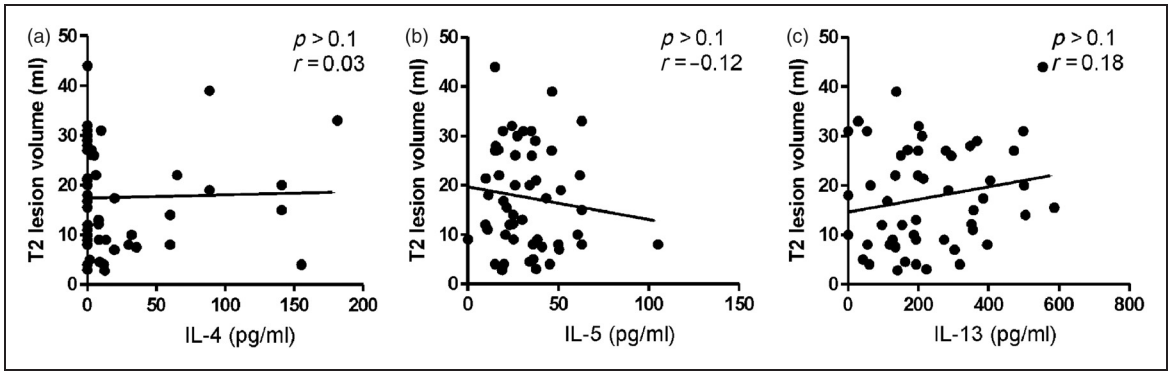

Figure 2. Correlation between TH2 cytokines and brain lesion load. A–C. The scatter plots show no correlation between cerebrospinal fluid levels of IL-4 (A), IL-5 (B), IL-13 (C) and lesion volume evaluated at magnetic resonance imaging.

although not statistically significant, correlation between IL-13 CSF levels and better performance at the MSFC ($n = 23$, $r = 0.40$, $p = 0.06$). A lack of correlation was conversely found between the other TH2 cytokines and MSFC scoring (IL-4: $n = 23$, $r = –0.06$, $p > 0.1$; IL-5: $n = 23$, $r = –0.07$, $p > 0.1$) (Figure 5A, 5C, 5E).

Remitting MS patients included in the study had a low mean EDSS value (1.0 ± 0.9, range 0–3.0). No significant correlation was found between the EDSS and any of the cytokines analysed (IL4: $n = 23$, $r = –0.01$, $p > 0.1$; IL-5: $n = 23$, $r = 0.05$, $p > 0.1$; IL-13: $n = 23$, $r = –0.3$, $p = 0.1$) (Figure 5B, 5D, 5F).

Correlation between TH2 cytokines and intracortical synaptic excitability

The modulation of synaptic transmission by soluble mediators of inflammation is likely to impact on neuronal survival, as suggested in a number of in vitro studies

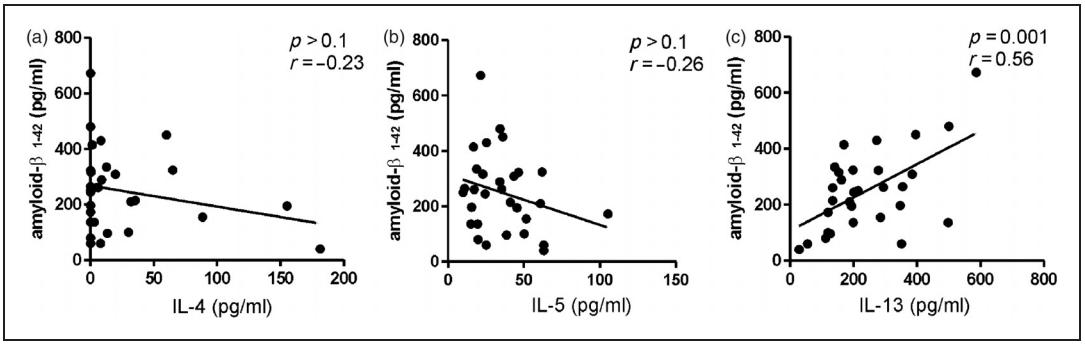

Figure 3. Correlation between TH2 cytokines and a soluble marker of neurodegeneration. A–C. The scatter plots show that neither IL-4 (A) nor IL-5 (B) were correlated with amyloid- $\beta_{1\text{-}42}$ in the cerebrospinal fluid (CSF) of patients with MS. A significant correlation was found between IL-13 and amyloid-$\beta_{1\text{-}42}$ CSF contents (C).

Table 2. Correlation analysis between TH2 cytokines and optical coherence tomography parameters in patients with multiple sclerosis

	IL-4	IL-5	IL-13
RNFLt average	$p = 0.14$, $r = -0.36$	$p = 0.95$ $r = -0.01$	$p < 0.001$ $r = 0.74$
RNFLt TQ	$p = 0.35$ $r = -0.24$	$p = 0.96$ $r = -0.01$	$p < 0.001$ $r = 0.70$
RNFLt SQ	$p = 0.22$ $r = -0.30$	$p = 0.81$ $r = 0.06$	$p = 0.02$ $r = 0.54$
RNFLt NQ	$p = 0.10$ $r = -0.40$	$p = 0.76$ $r = -0.08$	$p = 0.05$ $r = 0.47$
RNFLt IQ	$p = 0.22$ $r = -0.30$	$p = 0.92$ $r = -0.02$	$p < 0.001$ $r = 0.47$
MV	$p = 0.20$ $r = 0.32$	$p = 0.21$ $r = 0.30$	$p < 0.001$ $r = 0.78$

RNFLt, retinal nerve fibre layer average thickness; TQ, temporal quadrant; SQ, superior quadrant; NQ, nasal quadrant; IQ, inferior quadrant; MV, macular volume.

demonstrating correlation between the pro-excitatory and pro-degenerative effects of TH1 cytokines.[4,8–10] Thus, in a subgroup of 28 patients who gave consent to the ppTMS procedure, we explored the possible correlations between IL-4, IL-5 and IL-13 and both excitatory and inhibitory cortical transmission. No correlation was found between IL-4 or IL-5 CSF levels with SICI, ICF, LICI and SICF ($p > 0.05$ for both IL-4 and IL-5 at each ISI explored). In contrast, a significant correlation was found between IL-13 and SICI evoked at ISI 5 ($r = -0.54$, $p = 0.003$) and 6 ms ($r = -0.41$; $p = 0.03$), suggesting that IL-13 might exert neuroprotective effects in MS brains by favouring GABAA over glutamate transmission in MS brains (Figure 6).

Discussion

Neurodegeneration accompanies inflammation from the early stages of MS, and involves not only overtly demyelinated areas, but also normal-appearing white and grey matter.[1,52,53] Pro-inflammatory cytokines released by activated T lymphocytes and microglia are plausible mediators of the neurodegenerative damage in MS, but a role for anti-inflammatory cytokines in the control of this process has also been postulated.[11–14,54–56] Although increased production of IL-5 and IL-13 due to immunomodulatory therapy has been reported,[57–60] to date the demonstration of fluctuation of TH2 cytokines with disease activity of MS is controversial.[61–63]

In the present study, we provided some evidence that IL-13, a main anti-inflammatory cytokine released from TH2 lymphocytes, may play a role in the control of the neurodegenerative damage of MS, because CSF levels of this cytokine were positively associated with amyloid-$\beta_{1\text{-}42}$ levels, with neuronal preservation at the OCT and with functional outcomes. Conversely, the levels of IL-13 were unrelated to the extent of CNS demyelination and inflammatory disease activity. On the other hand, the levels of the other TH2 cytokines, IL-4 and IL-5, seemed to be associated to acute demyelination in the CNS of patients with MS, without any effect on the neuronal compartment.

Amyloid-$\beta_{1\text{-}42}$ levels are reduced in patients with MS concomitantly with tissue destruction during the active phases of the disease.[28,64] Also in Alzheimer's disease, amyloid-$\beta_{1\text{-}42}$ CSF levels are low compared with control subjects, likely because deposition in the brain parenchyma reduces its CSF levels.[65] Importantly, IL-1β, an important pro-inflammatory cytokine involved in MS pathophysiology, accelerates amyloid-β deposition in the cerebral cortex,[66,67] while anti-inflammatory cytokines enhance the activity of amyloid-β- degrading enzymes, thus favouring amyloid-β tissue deposit clearance.[68] Our results are, therefore, in good agreement with the idea that the anti-inflammatory cytokine IL-13 prevents amyloid-β tissue deposition and neuronal damage, because higher amyloid-$\beta_{1\text{-}42}$ levels were found in those subjects with higher IL-13 CSF levels.

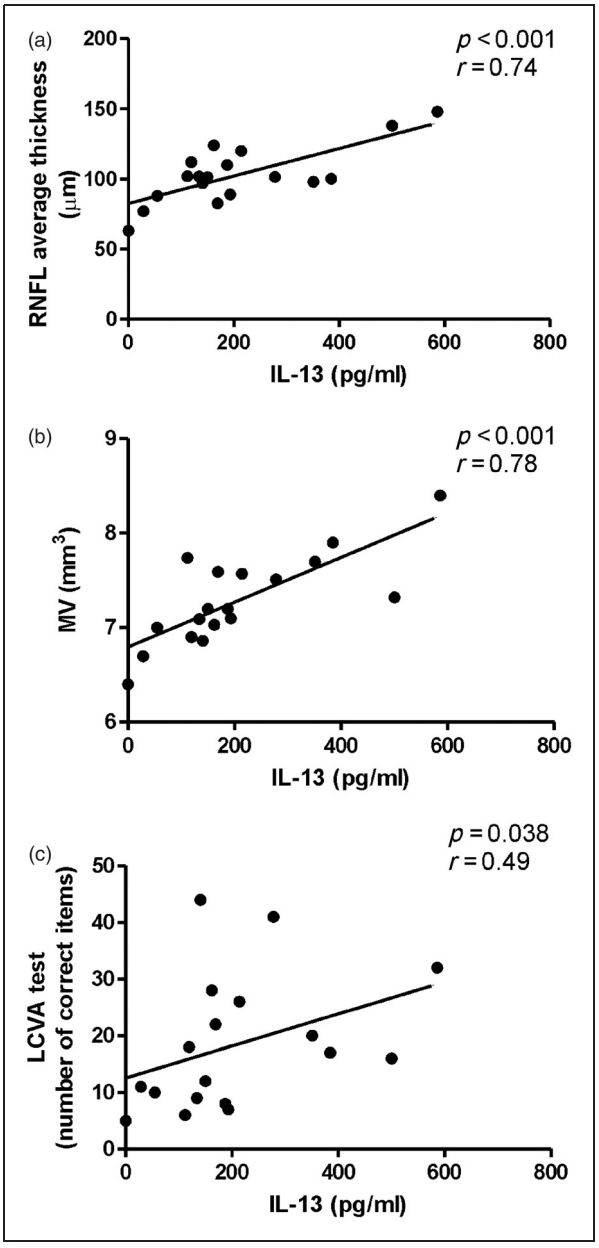

Figure 4. Correlation between IL-13 and neurodegenerative damage. A, B. The graphs show that IL-13 cerebrospinal fluid (CSF) levels were positively associated with axonal and neuronal preservation evaluated by optical coherence tomography in patients with multiple sclerosis, by retinal nerve fibre layer (RNFL) thickness (A) and macular volume (MV) (B) measures. C. IL-13 CSF levels were significantly related to better performance in the low-contrast visual acuity (LCVA) test.

It is increasingly accepted that retinal alterations in MS patients accurately model the mechanisms of neurodegeneration in MS, and that MV and RNFL thickness, obtained by OCT scans, are reliable measures of the integrity of, respectively, both neurons and their axonal projections within the retina.[32] In fact, a close relationship has been found between RNFL thickness and brain atrophy and tissue damage evaluated at MRI.[30,31] Furthermore, patients with more progressive MS courses have more substantial RNFL loss,[69] again confirming that the alterations of RNFL thickness mirror those occurring in the brains of MS subjects. Based on these considerations, therefore, the evidence that patients with higher IL-13 contents in the CSF have less alterations at the OCT is in good agreement with the idea that this anti-inflammatory cytokine exerts a diffuse neuroprotective effect in MS. According with this finding, IL-13 production by regulatory T cells protects against EAE and prevents axonal injury.[70,71] Recently, IL-13 has been shown to induce CD200 receptor,[72,73] providing a putative mechanism of neuroprotection in MS. In fact, CD200 and its receptor, homologous membrane glycoproteins that belong to the immunoglobulin superfamily, have been found to be involved not only in the control of immune response but also in neuron-glia interaction in the CNS and retina.[74–76] In particular, CD200-deficient mice have enhanced susceptibility to EAE and worse neuronal cell death,[77] whereas mice with inherently increased levels of CD200 have milder clinical disease, and show increased neuroprotection.[78] Dysregulation of CD200–CD200 receptor signalling has been also found to occur in MS[76,79] and in other neurodegenerative disorders, such as in Alzheimer's disease[72] and Parkinson's disease.[80] These findings suggest that IL-13 could protect the vulnerable neurons in the course of MS by modulating CD200–CD200 receptor signalling. The higher content of this cytokine in the CSF of MS patients with respect to control subjects may be the result of a compensatory mechanism during inflammatory neurodegeneration.

The levels of IL-13 in the CNS of the patients with MS were related to better performances in the LCVA test and MSFC scoring, thus providing beneficial effects not only on neuronal structural findings but also on clinical functional outcomes. The fact that we found a stronger correlation with disability measured by MSFC than by EDSS could be explained because of the better sensitivity of the former scoring protocol in detecting subclinical axonal dysfunction. In line with this, in studies measuring disability and neurodegeneration, the MSFC correlated better than the EDSS with the amount of brain atrophy.[81,82] Notably, MS patients at relatively early stages of the disease (and consequent low EDSS scores) were included in the present study, because CSF withdrawal was performed for diagnosis purposes. Further studies on different stages of MS may be important to strengthen the reported neuroprotective role of IL-13.

Studies in animal models have revealed that alterations of synaptic transmission are important correlates

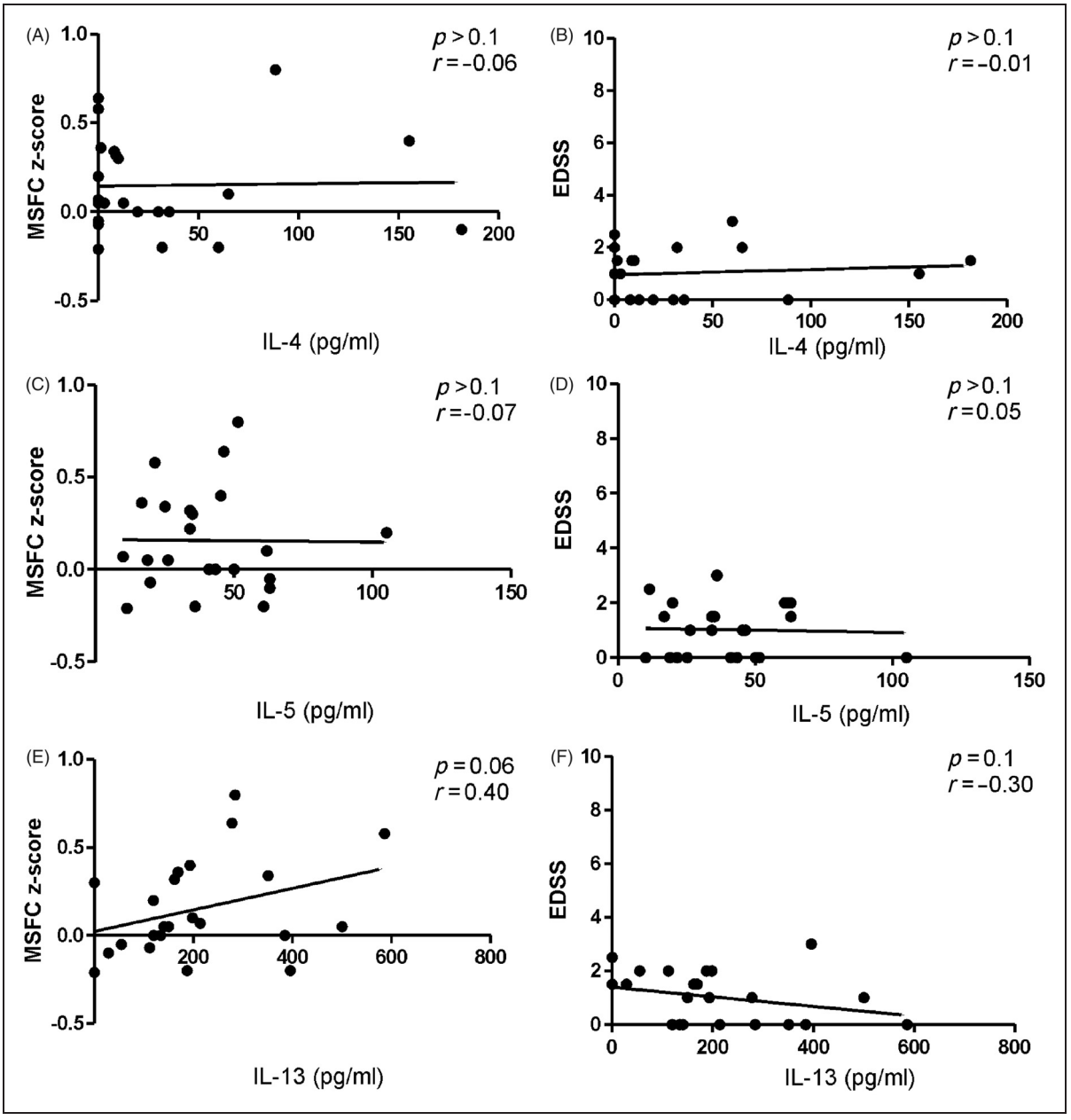

Figure 5. Correlation between TH2 cytokines and disability. A,B. IL-4 cerebrospinal fluid (CSF) levels were not related to disability assessed by both Multiple Sclerosis Functional Composite (MSFC) (A) and Expanded Disability Status Scale (EDSS) (B). C,D. IL-5 CSF levels were not related to disability assessed by both MSFC (C) and EDSS (D). E. A positive correlation, although not statistically significant, was found between IL-13 CSF levels and MSFC Z-score. F. A tendency towards a negative correlation was found between IL-13 levels and disability assessed by EDSS.

of neuronal injury,[83] and exacerbated neuronal excitation by imbalance between glutamate and GABA transmission has been postulated to contribute substantially to neuronal damage in MS. Glutamate, in fact, increases in the brain[21,22] and in the CSF of patients with MS,[84,85] and pharmacological blockade of glutamate receptors ameliorates MS[86] and EAE clinical course.[87,88] EAE is also attenuated by pharmacological potentiation of GABA transmission[89] and, in MS patients, GABA CSF levels are reduced,[20] along with a significant loss of GABAergic interneurons in the normal-appearing grey matter and in the motor cortex.[90,91] Of note, defective GABAergic transmission within the motor cortex has also been postulated in patients with active MS on the basis of neurophysiological findings with ppTMS,[92] and in the present study we have shown that GABAA-mediated cortical inhibition is significantly more pronounced in patients

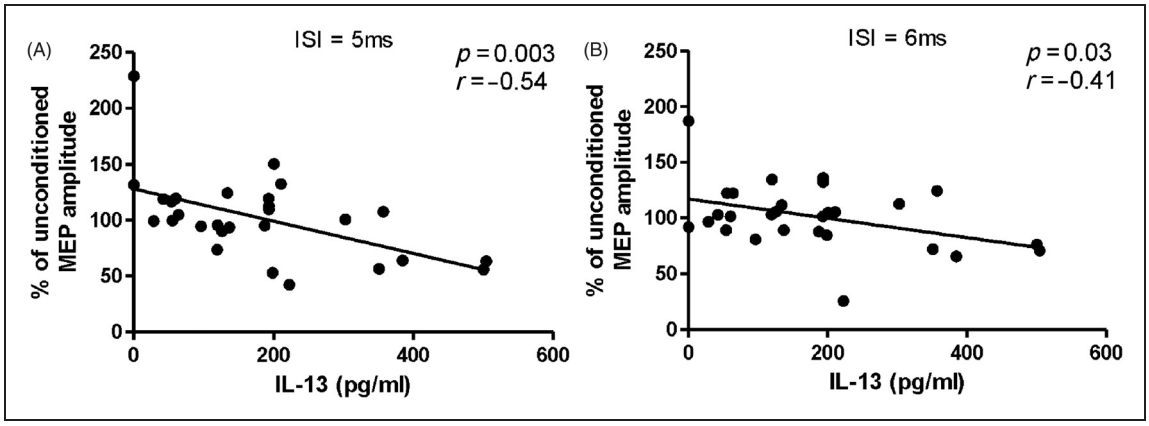

Figure 6. Correlation between TH2 cytokines and intracortical synaptic excitability. A, B. The scatter plots show the positive correlation found between IL-13 cerebrospinal fluid levels and intracortical inhibition evaluated at both 5 ms (A) and 6 ms (B) interstimulus interval (ISI) by means of paired pulse transcranial magnetic stimulation.

with high IL-13 levels in the CSF, as expected for a neuroprotective effect of this anti-inflammatory cytokine. Interestingly, glutamate-mediated excitotoxicity has been implicated in the mechanism of neurodegeneration also in experimental optic neuritis,[93] again supporting the common pathophysiology of neuronal damage in the brain and in the retina of MS patients. In the present study only SICI at 5 and 6 ms ISI correlated with IL-13 levels, but not at ISI 2 and 3 ms. Kujirai and co-authors[39] showed that 1–3 ms ISIs produced the maximal inhibitory effect of ppTMS, while at 5 and 6 ms ISI less suppression was produced. Thus, we may argue that at 2 and 3 ms ISI, inhibition may have reached its limit and may not be, therefore, further increased, while at 5 and 6 ms ISI, being the magnitude of submaximal inhibition, a potentiating effect of IL-13 may still be recordable. Of note, we have previously demonstrated that CSF levels of amyloid-$\beta_{1\text{-}42}$, here related to central levels of IL-13, interfere with the induction of long-term potentiation-like cortical plasticity explored with TMS, without affecting basal transmission in MS subjects.[28]

In conclusion, the present investigation provides molecular, imaging, and physiological evidence of the involvement of IL-13 in the modulation of neuronal integrity and synaptic function in patients with MS, suggesting that pharmacological treatments able to up-regulate IL-13 production by TH2 lymphocytes might have not only immunomodulatory but also neuroprotective effects in MS.

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Conflict of interest statement

The authors declare that they have no conflicts of interest.

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