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Gut bacteria *Akkermansia* elicit a specific IgG response in CSF of patients with MS

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MS is a chronic inflammatory disease of the CNS involving T cell and B cell responses. Recently, several studies have described modifications of specific bacterium abundances of gut microbiota in patients with remitting–relapsing MS compared with healthy individuals (see for review[1]). This was often associated with an increase in *Akkermansia muciniphila* bacteria. In experimental autoimmune encephalomyelitis, transfer of gut microbiota from patients with MS to mice induced proinflammatory responses and exacerbation of the disease, whereas microbiota from healthy volunteers (HVs) were less inflammatory.[2,3] Because bacteria in the gut modulate immune responses, we assessed the antibody production against *A muciniphila* in patients with MS. In CSF, levels of anti-*A muciniphila* immunoglobulin G (IgG) were increased in patients with MS compared with controls, whereas no difference was found for levels of IgG against *Escherichia coli*, *Fusobacterium necrophorum*, *Acinetobacter baumannii*, *Prevotella melaninogenica*, and *Bacteroides fragilis*.

Methods

Patients with relapsing–remitting MS (RRMS, n = 62) were enrolled in the neurology department of CHU de Nantes. Sex and age-matched patients (HVs, n = 41), patients with noninflammatory neurologic disease (e.g., suffering from sudden headaches or idiopathic intracranial hypertension) (noninflammatory neurologic disease [NIND], n = 23), and patients with inflammatory neurologic disease (peripheral neuropathy, brain lymphoma) (inflammatory neurological disease [IND], n = 10) were used for comparison. Informed written consent was obtained from all the patients before any study-related procedure was performed. Patients had not received any disease-modifying drugs before the sampling.

IgG concentration was measured with an immunonephelometric assay performed using a Beckman Immage Analyzer (Beckman Coulter). To detect antibody against bacteria, ELISA tests were performed using serum and CSF samples. Briefly, bacteria lysates from *A muciniphila*, *F necrophorum*, *A baumannii*, *P melaninogenica*, and *E coli* were coated on plates (Nunc) at 1 μg/mL of proteins in phosphate buffer saline (PBS). Bovine serum albumin (Sigma Aldrich) at 1% in PBS was used for blocking. Patient samples were incubated for 2 hours at 37°C in PBS (dilutions 1/100 for serum, 1/10 for CSF) and 1% bovine serum albumin. Antihuman IgG antibodies coupled with horseradish peroxidase (Bethyl Laboratories) at 1/5,000, 1 hour at 37°C, were used. The reaction with the substrate (3,3',5,5'-Tetramethylbenzidine, BD Biosciences) was stopped with sulfuric acid (0.18M, Sigma Aldrich). Plates were read at 450 nm using a Spark 10M multimode

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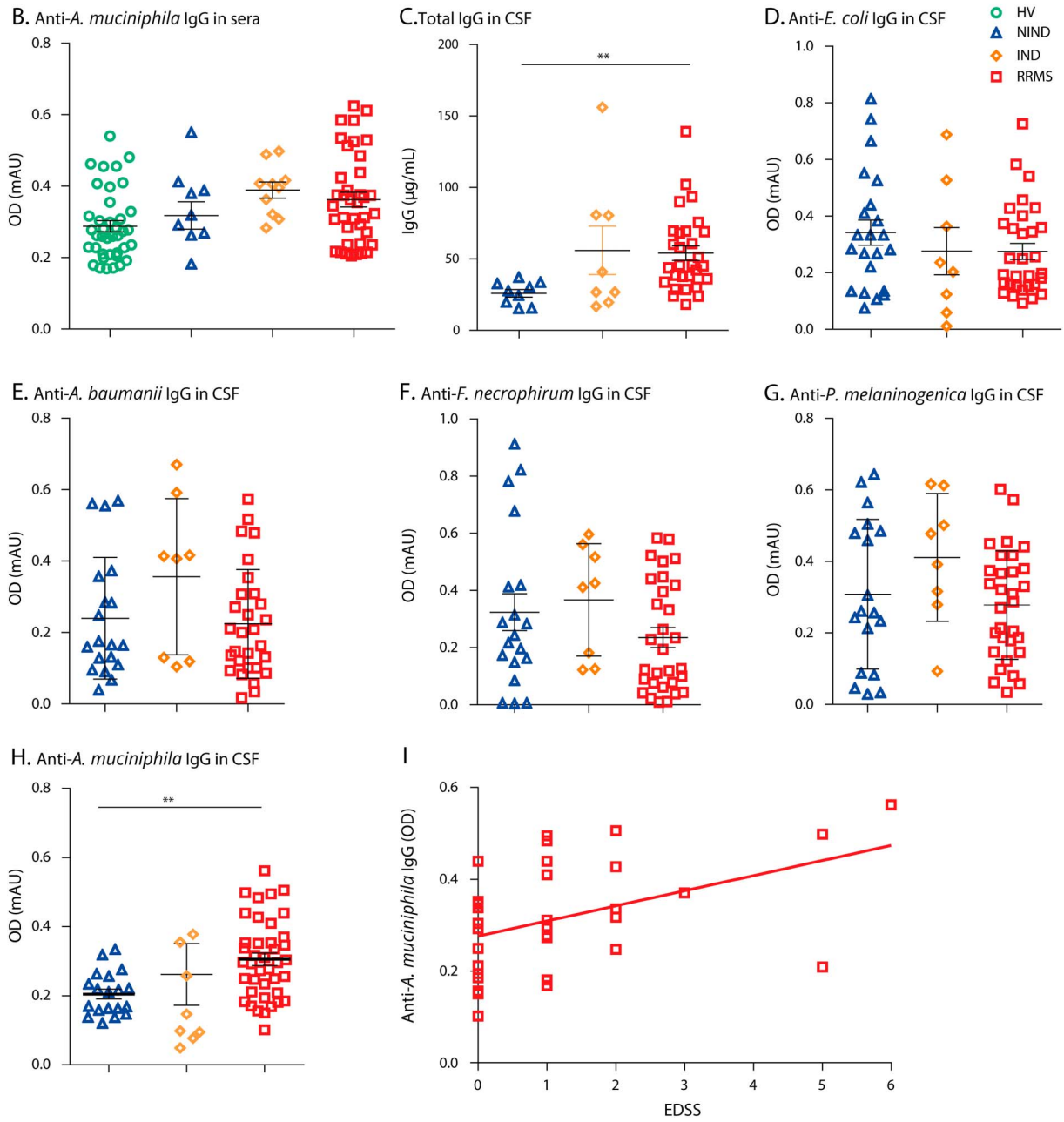
Figure Increased levels of Anti-Akkermansia IgG in RRMS CSF and correlation with the EDSS score

A. Characteristics of patients

	NIND (n = 23)	IND (n = 10)	HV (n = 41)	RRMS (n = 62)
Age (years), median (IQR)	49.5 (36.0–62.5)[a]	45.8 (37.0–53.0)	36.0 (27.0–44.5)	36.0 (28.0–44.0)
Female sex, n (%)	14 (60.8)	8 (80.0)	28 (68.3)	49 (79.1)
Disease duration (years), median (IQR)				3.0 (0.3–10)
EDSS score, median (IQR)				1.5 (0.0–2.5)



(A) Clinical characteristics of patients are listed in Table A. Patients with RRMS; patients with noninflammatory neurological disease of the CNS (NIND), patients with IND, and HVs; EDSS, median ± IQR, [a]$p < 0.01$ NIND vs HV, and RRMS. (B) Levels of anti-*Akkermansia muciniphila* IgG in sera from patients with RRMS, NIND, IND, and HV. (C) Total IgG concentrations in CSF from patients with RRMS, NIND, and IND. (D) Levels of anti-*Escherichia coli* IgG in CSF from patients with RRMS, NIND, and IND. (E) Levels of anti-*Acinetobacter baumannii* IgG in CSF. (F) Levels of anti-*Fusobacterium necrophorum* IgG in CSF. (G) Levels of anti-*Prevotella melaninogenica* IgG in CSF. (H) Levels of anti-*A muciniphila* IgG in CSF. Mean ± standard error. **$p < 0.01$. (I) Correlation between anti-*A muciniphila* IgG in CSF from patients with RRMS and EDSS score (Spearman's rank correlation rho = 0.36, $p = 0.027$). EDSS = Expanded Disability Status Scale; HV = healthy volunteer; IND = inflammatory neurological disease; IQR = interquartile range; NIND = noninflammatory neurological disease; RRMS = relapsing–remitting MS.

microplate reader (Tecan). The mean values of optical density were compared using the Mann-Whitney test and analysis of variance with Dunn's multiple comparisons test for more than 2 groups. Aberrant values for biological variables were determined by a Dixon test and excluded.

Results

The levels of anti-*A muciniphila* IgG were measured by using ELISA in sera and appeared equivalent in patients with RRMS, NIND, IND, and HV (figure, B). The IgG levels of anti-*E. coli*, *F. necrophorum*, *A baumannii*, and *P melaninogenica* were also similar in the tested groups (data not shown). As expected and previously described, the total IgG concentrations were increased in CSF from patients with RRMS compared with those of NIND ($p < 0.01$, figure, C). There was no difference in the IgG levels against *E coli*, *F necrophorum*, *A baumannii*, and *P melaninogenica* in CSF from the 3 groups (figure, D–G). We were able to reveal an increased IgG reaction against *A muciniphila* in patients with RRMS compared with those with NIND and IND ($p < 0.005$, figure, H). There was no correlation between the total IgG concentration and levels of anti-*A muciniphila* IgG in CSF (data not shown). Strikingly, the levels of anti-*A muciniphila* correlated with Expanded Disability Status Scale score in RRMS (rho = 0.36, $p = 0.027$, figure, I).

Discussion

This is the first study to display that the levels of IgG against *A muciniphila* are increased in CSF of patients with RRMS compared with control samples. Interestingly, anti-*E Coli* IgG levels in CSF were equivalent in patients with RRMS and controls, revealing a specific response against a particular bacterium. An increase in *A muciniphila*, *Fusobacterium*, and *Acinetobacter* bacteria and a decrease in *Prevotella* have been shown in the gut microbiota of patients with RRMS. However, we found that, although the CSF samples also exhibited an increase in anti-*A muciniphila* IgG levels, no such modification was detected in the blood. This CSF-localized specific antibody signature suggests an intrathecal secretion of specific B cells retained in the CNS compartment, which may have migrated from the gut. B cells were indeed found while draining cervical lymph nodes of patients with MS.[4] These B cells were able to populate the CNS in this inflammatory context. Moreover, in a mouse model, plasma cells specific for commensal bacteria were diminished in the gut and circulate to the CNS, producing in situ immunoglobulins.[5] The local role of anti-*A muciniphila* IgG in MS pathology remains yet to be elucidated. Antibodies could be also elicited or act through cross-reactivity/molecular mimicry to myelin components as was described recently for T cell responses to some microbiota-derived peptides.[6] Thus, along with previous lines of evidence for gut microbiota modifications in MS, our present findings highlight a CNS-specific antibacterial antibody response in patients with RRMS.

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Disclosure

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Amélie Vallino, MD	Center of Research in Transplantation and Immunology, Nantes, France	Major role in the acquisition of data, Interpreted the data
Amélie Dos Santos, MS	Center of Research in Transplantation and Immunology, Nantes, France	Major role in the acquisition of data, Interpreted the data
Camille V. Mathé	Center of Research in Transplantation and Immunology, Nantes, France	Major role in the acquisition of data, Interpreted the data
Alexandra Garcia	Center of Research in Transplantation and Immunology, Nantes, France	Major role in the acquisition of data
Jeremy Morille, MS	Center of Research in Transplantation and Immunology, Nantes, France	Major role in the acquisition of data
Emilie Dugast, PhD	Center of Research in Transplantation and Immunology, Nantes, France	Major role in the acquisition of data
Sita P. Shah, MS	Center of Research in Transplantation and Immunology, Nantes, France	Revised the manuscript for intellectual content
Geneviève Héry-Arnaud, PharmD, PhD	CHU de Brest, France	Major role in the acquisition of data
Charles-Antoine Guiloux, MS	University of Brest	Major role in the acquisition of data
Patrick James Gleeson, MD	Center of Research on Inflammation, Paris, France	Major role in the acquisition of data
Renato Costa Monteiro, MD, PhD	Center of Research on Inflammation, Paris, France	Major role in the acquisition of data; revised the manuscript for intellectual content

Continued

Appendix *(continued)*

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Jean-Paul Souillou, MD	Center of Research in Transplantation and Immunology, Nantes, France	Revised the manuscript for intellectual content
Jean Harb, PhD	Center of Research in Transplantation and Immunology, Nantes, France	Revised the manuscript for intellectual content
Edith Bigot-Corbel, PharmD, PhD	CHU de Nantes, France	Major role in the acquisition of data
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Arnaud B. Nicot, PhD	Center of Research in Transplantation and Immunology, Nantes, France	Interpreted the data; revised the manuscript for intellectual content

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Laureline Berthelot, PhD	Center of Research in Transplantation and Immunology, Nantes, France	Design and conceptualized study; analyzed the data; drafted the manuscript for intellectual content

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