ELSEVIER
Journal of Neuroimmunology xx (2007) xxx–xxx
Journal of Neuroimmunology
www.elsevier.com/locate/jneuroim

Short communication

Helicobacter pylori infection is a potential protective factor against conventional multiple sclerosis in the Japanese population

Wei Li, Motozumi Minohara, Jen Jen Su, Takeshi Matsuoka, Manabu Osoegawa, Takaaki Ishizu, Jun-ichi Kira *

Department of Neurology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, Fukuoka 812–8582, Japan

Received 19 September 2006; received in revised form 7 December 2006; accepted 19 December 2006

Abstract

Persistent *Helicobacter pylori* (*H. pylori*) infection is a chronic inflammatory stimulus to hosts with an inverse correlation to atopic disorders. In this study, a total of 105 consecutive multiple sclerosis (MS) patients were divided into 52 opticospinal MS (OSMS) and 53 conventional MS (CMS), and their sera, along with those from 85 healthy controls (HC), were examined by an enzyme-linked immunosorbent assay using antibodies against *H. pylori*. *H. pylori* seropositivity was significantly lower in patients with CMS (22.6%) compared with HC (42.4%) and patients with OSMS (51.9%) ($p=0.0180$ and $p=0.0019$, respectively). In patients with CMS, *H. pylori* seropositivity showed a significant inverse association with mean EDSS score and fulfillment of McDonald MRI criteria for space (OR=0.61, $p=0.0344$ and OR=0.11, $p=0.0297$). These findings suggest that *H. pylori* infection is a protective factor against CMS in Japanese.

Keywords: Multiple sclerosis; *Helicobacter pylori*; Japanese; Opticospinal MS

1. Introduction

Multiple sclerosis (MS) is the most common inflammatory demyelinating disease of the central nervous system (CNS) in humans. MS in Asians is characterized by a low prevalence rate and selective involvement of the optic nerve and spinal cord. Opticospinal MS (OSMS) is observed in 15–40% of Japanese MS patients, with the remainder having features that are similar to those of MS in Caucasians (conventional or classical MS; CMS) (Kira, 2003). Recently, a sharp rise in the ratio of CMS to OSMS in the Japanese population was reported, especially among those born after Japan's period of rapid economic growth, together with an increase in the overall prevalence of MS (Kira et al., 1999; Kira, 2003). Although the etiology of MS is unknown, the environment during childhood has a critical effect on the susceptibility to MS (Compston, 1997). Therefore, environmental changes accompanied with Japan's Westernization may have altered the incidence and phenotypes of MS in Japanese.

Helicobacter pylori (*H. pylori*) are gram-negative microaerophilic bacteria that reside in the stomachs of more than 50% of the entire human population (Blaser, 1993). *H. pylori* infection has been reported to have an inverse correlation with the prevalence of atopic disorders (McCune et al., 2003), which is consistent with the hygiene hypothesis; that is, that an early childhood infection can suppress allergic disorders (Cremonini and Gasbarrini, 2003). The present study was designed to reveal the prevalence of *H. pylori* infection in each MS subtype in the Japanese population.

2. Subjects and methods

2.1. Subjects

A total of 105 consecutive patients (24 men and 81 women) with MS, diagnosed at the Department of Neurology,

* Corresponding author. Tel.: +81 92 642 5340; fax: +81 92 642 5352.
E-mail address: kira@neuro.med.kyushu-u.ac.jp (J. Kira).

0165-5728/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jneuroim.2006.12.010

Please cite this article as: Li, W. et al. *Helicobacter pylori* infection is a potential protective factor against conventional multiple sclerosis in the Japanese population. Journal of Neuroimmunology (2007), doi:10.1016/j.jneuroim.2006.12.010

Kyushu University Hospital according to the criteria of McDonald et al. (2001), were enrolled in this study. The age at examination was 46.9 ± 13.2 years (mean ± SD; range: 14 to 73 years) and the age at disease onset was 32.9 ± 14.2 years (range: 9 to 70 years). There were no obvious gastropathy symptoms, such as gastric and duodenal ulcer, at the time of blood sampling. Patients were classified clinically into two subtypes: OSMS or CMS, as described previously (Kira et al., 1996). OSMS diagnosed according to the criteria for OSMS (Kira, 2003). Briefly, 52 patients whose clinically estimated main lesions were confined to both optic nerve and spinal cord were classified as OSMS. These patients had no clinical evidence of disease in either the cerebrum or cerebellum; however, minor brainstem signs, such as transient double vision and nystagmus, were acceptable. The remaining 53 patients had multiple involvement of the CNS, including the cerebrum, cerebellum and brainstem, and were classified as CMS. In addition, eighty-five healthy subjects (21 men and 64 women) were enrolled as healthy control (HC) subjects. Their average age at sampling was 43.5 ± 12.6 years (range: 21 to 64 years). The disability status of patients was scored by one of the authors (TM) using Kurtzke's Expanded Disability Status Scale (EDSS) score (Kurtzke, 1983).

2.2. Anti-H. Pylori antibody assay

The presence in the serum of antibody against *H. pylori* was determined by an ELISA using SMITEST ELISA helicobacter (Chemicon, Australia) according to the manufacturer's instructions. A measure of 50 U/ml was set as the cut-off value for this assay, such that any reading greater than this was considered as a positive *H. pylori* infection.

2.3. Magnetic resonance imaging

All MR studies were performed using a 1.5 T Magnetom Vision and Symphony (Siemens Medical Systems, Erlangen, Germany) MRI system (Su et al., 2006). Brain and spinal cord MRI were evaluated independently by two of the authors (JJS and TM) who were naive to the diagnoses. Brain MRI lesions were evaluated according to McDonald's MRI criteria for MS (McDonald et al., 2001). Spinal cord lesions longer than three vertebral segment lengths were considered to be longitudinally extensive spinal cord lesions (LESCL).

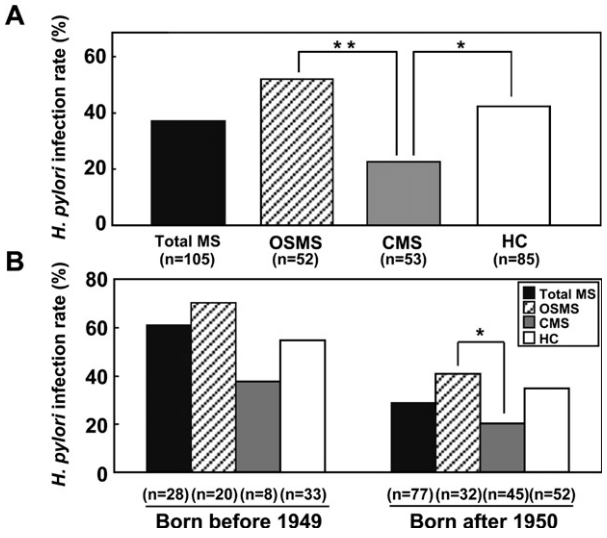

Fig. 1. Frequency of *H. pylori* seropositivity in MS patients and healthy controls. (A) *H. pylori* seropositivity was significantly lower in CMS patients, as compared with OSMS patients and healthy controls (*, $p<0.05$; **, $p<0.005$). (B) *H. pylori* seropositivity was significantly lower in CMS patients than in OSMS patients among those born after 1950 (*, $p<0.05$).

2.4. Statistical analysis

Statistical analyses of ages at onset, blood sampling and final follow-up, disease duration and EDSS scores were initially performed using the Kruskal–Wallis *H* test. When statistical significance was found, the Mann–Whitney *U* test was used to determine the statistical differences between each subgroup. Uncorrelated *p*-values were corrected by multiplying by the number of comparisons (Bonferroni–

Table 1
Demographic features of the subjects

	Total MS (N=105)	OSMS (N=52)	CMS (N=53)	HC (N=85)
Male : Female	24 : 81 (1 : 3.38)	9 : 43 (1 : 4.78)	15 : 23 (1 : 2.53)	21 : 64 (1 : 3.05)
Age at onset (years)[a]	32.9±14.2	36.5±15.0 *	29.4±12.5	NA
Age at blood sampling (years)[a]	41.2±13.2	44.9±13.9 *	37.6±11.5 *	43.5±12.6
Age at final follow-up (years)[a]	46.9±13.2	50.1±14.2 *	43.7±11.4	NA
Disease duration at blood sampling (months)[a]	96.8±89.7	97.3±89.2	96.2±91.0	NA
Disease duration at final follow-up (months)[a]	144.3±102.9	148.5±100.3	140.1±106.1	NA
EDSS scores at final follow-up[a]	4.3±2.6	4.4±2.6	4.2±2.6	NA
Fulfilling McDonald MRI criteria (%)	37.2	19.6 *	57.5	NA
LESCL (%)	35.5	47.9 *	22.2	NA

EDSS: Kurtzke's Expanded Disability Status Scale; LESCL: longitudinally extensive spinal cord lesion; NA: not applicable.
Mean ± SD, * $p < 0.05$.

Please cite this article as: Li, W. et al. *Helicobacter pylori* infection is a potential protective factor against conventional multiple sclerosis in the Japanese population. Journal of Neuroimmunology (2007), doi:10.1016/j.jneuroim.2006.12.010

Table 2
Results of logistic regression analysis to predict *H. pylori*-seropositive versus seronegative in MS patients

Variables	Seropositive (N=39)	Seronegative (N=66)	Odds ratio	95% CI	p value
Gender, % female	76.9	77.3	0.59	0.20–1.73	0.3346
Mean age at onset, y (SD)	36.7 (14.8)	30.7 (13.5)	1.28	0.94–1.74	0.1206
Mean age at blood sampling, y (SD)	45.1 (13.6)	38.9 (12.5)	0.81	0.60–1.10	0.1729
Mean duration at blood sampling, y (SD)	8.4 (8.6)	7.9 (6.8)	1.02	0.99–1.04	0.1202
Subtype, % CMS	30.8	62.1	0.29	0.12–0.71	0.0067

CI: confidence interval.

Dunn's correction). The differences between two proportions were tested for significance by Fisher's exact test. Logistic regression analysis was performed to assess the association of MS with the *H. pylori* antibody-positive group and, within each MS subtype, the association of the *H. pylori* positivity with clinical and MRI parameters. In all assays, significance was defined as $p<0.05$.

3. Results

3.1. Demographic features of each subtype of MS

The demographic features of the patients are summarized in Table 1. The ages at onset, at blood sampling and at final follow-up were significantly higher in OSMS patients than in CMS patients ($p=0.0282$, $p=0.0246$, $p=0.0345$, respectively). The disease durations at the time of blood sampling and at final follow-up, and the EDSS at final follow-up, did not differ significantly between OSMS and CMS subtypes. The frequency of patients who fulfilled the McDonald MRI criteria for space was significantly higher in the CMS group than in the OSMS group (57.5% vs. 19.6%, $p=0.0003$), whereas the frequency of LESCL was higher in the OSMS group than in the CMS group (47.9% vs. 22.2%, $p=0.0097$).

3.2. Frequency of H. Pylori seropositivity in MS patients and healthy controls

The frequency of *H. pylori* seropositivity did not differ significantly between MS patients collectively (39/105, 37.1%) and HC (36/85, 42.4%) (Fig. 1). When analyzed separately by clinical phenotype, *H. pylori* seropositivity was significantly lower in CMS patients (12/53, 22.6%), as compared with OSMS patients (27/52, 51.9%) and HC ($p=0.0019$ and $p=0.0180$, respectively). Furthermore, when analyzed separately by year of birth, in patients born after 1950, *H. pylori* seropositive rate was significantly lower in CMS patients (9/45, 20%) than OSMS patients (13/32, 40.6%; $p=0.0483$). On the other hand, in patients born before 1950, although the rate of seropositivity in CMS patients was again about half that of OSMS patients (37.5% vs. 70%), the rates of seropositivity did not differ significantly between the groups, presumably because of the small sample size. Regardless of a birth-date before or after 1950, *H. pylori* seropositivity was lower in patients with CMS compared with HC; however, the difference did not reach statistical significance.

3.3. Relationship between H. Pylori seropositivity and MS subtype

The MS patients were divided into *H. pylori* seropositive and seronegative groups and the demographic parameters, shown in the methods and Table 2, were compared between the two groups by logistic regression analysis. Among the variables examined, *H. pylori* seropositivity showed a significant inverse association with CMS (OR=0.29 [95% CI=0.12 to 0.71], $p=0.0067$) (Table 2). Even when birth before or after 1950 was used as a variable instead of age at blood sampling, *H. pylori* infection had a significant inverse association with CMS (OR=0.321 [95%CI=0.13–0.78], $p=0.0124$)

Table 3

Variables	Seropositive (N=12)	Seronetgative (N=41)	Odds ratio	95% CI	p value
Results of logistic regression analysis to predict H. pylori-seropositive versus seronegative in CMS patients					
Mean duration at final follow-up, y (SD)	12.2 (10.1)	11.5 (8.6)	1.01	0.99–1.02	0.5623
Mean EDSS score, (SD)	3.08 (2.30)	4.59 (2.65)	0.61	0.39–0.96	0.0344
LESCL, %	18.2	23.5	10.50	0.68–163.27	0.0930
Fulfilling McDonald MRI criteria, %	27.3	69.0	0.11	0.02–0.81	0.0297
Variables	Seropositive (N=27)	Seronetgative (N=25)	Odds ratio	95% CI	p value
Results of logistic regression analysis to predict H. pylori-seropositive versus seronegative in OSMS patients					
Mean duration at final follow-up, y (SD)	13.0 (9.4)	11.7 (7.3)	1.00	0.99–1.01	0.4305
Mean EDSS score, (SD)	4.59 (2.57)	4.12 (2.66)	0.93	0.71–1.21	0.5702
LESCL, %	60.0	34.8	3.37	0.81–13.94	0.0939
Fulfilling McDonald MRI criteria, %	18.2	20.8	0.67	0.14–3.26	0.6155

EDSS: Kurtzke's Expanded Disability Status Scale; LESCL: longitudinally extensive spinal cord lesions; CI: confidence interval.

Please cite this article as: Li, W. et al. *Helicobacter pylori* infection is a potential protective factor against conventional multiple sclerosis in the Japanese population. Journal of Neuroimmunology (2007), doi:10.1016/j.jneuroim.2006.12.010

3.4. Relationship between H. Pylori seropositivity and clinical and MRI parameters in each MS subtype

Within the CMS group, among the clinical and MRI parameters examined by logistic regression analysis, *H. pylori* seropositivity demonstrated a significant inverse association with mean EDSS score (OR = 0.61 [95% CI = 0.39 to 0.96], $p = 0.0344$) and fulfillment of McDonald MRI criteria for space (OR = 0.11 [95%CI = 0.02 to 0.81], $p = 0.0297$) (Table 3). On the other hand, no variables were associated with *H. pylori* seropositivity in the OSMS group.

4. Discussion

The present study demonstrates that *H. pylori* infection is significantly lower in patients with CMS than in HC or patients with OSMS. The observation that *H. pylori* infection has a significant inverse association with CMS by logistic regression analysis further supports the notion that *H. pylori* is a protective factor against CMS in Japanese.

In Japanese, the prevalence of *H. pylori* infection is lower in the population born after 1950 than it is in those born before 1950 (Asaka et al., 1992). In our study, *H. pylori* seropositivity was also shown to be lower in subjects born after 1950 than in those born before 1950, in all subject groups. However, differences in *H. pylori* positivity among the three groups demonstrated a similar trend irrespective of date of birth. Furthermore, logistic regression analysis, using birth before or after 1950 as a variable, indicated that *H. pylori* positivity had an inverse association with CMS. There is only one report, in the Polish literature, indicating a lower frequency of *H. pylori* infection in MS as compared with controls (Wender, 2003). As CMS in Asians is considered to be the same as MS in Caucasians, our results are in accord with those of the Polish MS study. On the other hand, OSMS is characterized by a higher age at onset, marked female preponderance, frequent relapses, severe disability, fewer brain MRI lesions, LESCL, marked pleocytosis in the cerebrospinal fluid (CSF) and an absence of oligoclonal bands in the CSF; therefore, it is considered to have a distinct immune mechanism (Kira, 2003).

H. pylori infection is supposed to occur mainly before 2 years of age, primarily because the parietal cells that secrete gastric acids, which hamper the survival of *H. pylori*, are not well matured during infancy (Graham, 1991). Once acquired, the bacterium persists for years and decades (Graham, 1991). Thus, the difference in the frequency of *H. pylori* seropositivity suggests a distinction in the infectious environment during childhood. Therefore, the sanitary environment during childhood is thought to have been different between patients with CMS and those with OSMS; that is, a clean environment is associated with CMS and an infectious one is associated with OSMS.

Individuals infected with *H. pylori* were reported to be 30% less likely to have concomitant allergic diseases (McCune et al., 2003). A protective effect against allergy by *H. pylori* infection is considered to be the result of generic exposure to pathogens, rather than being due to the specific infection. A bacterial infection can influence the balance between T helper 1 (Th1) and T helper 2 (Th2) cells, augmenting a Th1 response while dampening the Th2 response. Thus, infection reduces the frequency of allergic disorders. However, the Th1/Th2 paradigm appears to be an oversimplification. Recent studies have reported that Th1 type autoimmune diseases, such as RA (Kero et al., 2001), Crohn's disease (Kero et al., 2001) and type 1 diabetes mellitus (Stene and Nafstad, 2001), are rather higher in patients that have airway allergies, a Th2 disease, and that both allergic disorders and autoimmune diseases are increasing in parallel. Such an increase occurs in younger generations in developed countries where good sanitation reduces the frequency of childhood infection. Therefore, Th1 and Th2 diseases coexist more frequently in atopic individuals raised in a clean environment. Less frequent bacterial infection is supposed to curtail the development of the immunoregulatory system in children, thereby perpetuating either allergic or autoimmune inflammation (Yazdanbakhsh et al., 2002). Recently, it has been shown that people with more siblings of a younger age have a reduced risk of MS (Ponsonby et al., 2005) suggesting that infection encountered early in life renders individuals resistant to MS. The results of our study appear to be consistent with this. Considering all of these observations together, a lower frequency *of H. pylori* infection might be a reflection of good sanitation, but it would seem to render individuals more susceptible to CMS. In CMS patients, the mean EDSS scores and brain MRI lesions fulfilling McDonald MRI criteria were inversely associated with *H. pylori* positivity, suggesting that *H. pylori* infection is a protective factor against the development of brain lesions and subsequent disability.

In summary, our study indicates a difference in *H. pylori* seropositivity between Japanese patients with OSMS and those with CMS. Although further study is required, these observations suggest that differences in childhood environment might exert distinct effects on the development of each MS subtype later in life.

Acknowledgements

This work was supported by grants from the Ministry of Education, Culture, Sports, Science and Technology, Japan. The Neuroimmunological Disease Research Committee and the Ministry of Health, Labor and Welfare, Japan, for Research on Brain Science.

References

Asaka, M., Kimura, T., Kudo, M., Takeda, H., Mitani, S., Miyazaki, T., Miki, T., Graham, D.Y., 1992. Relationship of *Helicobacter pylori* to serum pepsinogens in an asymptomatic Japanese population. Gastroenterology 102, 760–766.

Blaser, M.J., 1993. *Helicobacter pylori*: microbiology of a 'slow' bacterial infection. Trends Microbiol. 1, 255–259.

Please cite this article as: Li, W. et al. *Helicobacter pylori* infection is a potential protective factor against conventional multiple sclerosis in the Japanese population. Journal of Neuroimmunology (2007), doi:10.1016/j.jneuroim.2006.12.010

Compston, A., 1997. Genetic epidemiology of multiple sclerosis. J. Neurol. Neurosurg. Psychiatry 62, 553–561.

Cremonini, F., Gasbarrini, A., 2003. Atopy, *Helicobacter pylori* and the hygiene hypothesis. Eur. J. Gastroenterol. Hepatol. 15, 635–636.

Graham, D.Y., 1991. *Helicobacter pylori*: its epidemiology and its role in duodenal ulcer disease. J. Gastroenterol. Hepatol. 6, 105–113.

Kero, J., Gissler, M., Hemminki, E., Isolauri, E., 2001. Could TH1 and TH2 diseases coexist? Evaluation of asthma incidence in children with celiac disease, type 1 diabetes, or rheumatoid arthritis: a register study. J. Allergy Clin. Immunol. 108, 781–783.

Kira, J., Kanai, T., Nishimura, Y., Yamasaki, K., Matsushita, S., Kawano, Y., Hasuo, K., Tobimatsu, S., Kobayashi, T., 1996. Western versus Asian types of multiple sclerosis: immunogenetically and clinically distinct disorders. Ann. Neurol. 40, 569–574.

Kira, J., Yamasaki, K., Horiuchi, I., Ohyagi, Y., Taniwaki, T., Kawano, Y., 1999. Changes in the clinical phenotypes of multiple sclerosis during the past 50 years in Japan. J. Neurol. Sci. 166, 53–57.

Kira, J., 2003. Multiple sclerosis in the Japanese population. Lancet Neurol. 2, 117–127.

Kurtzke, J.F., 1983. Rating neurological impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology 33, 1444–1452.

McCune, A., Lane, A., Murray, L., Harvey, I., Nair, P., Donovan, J., Harvey, R., 2003. Reduced risk of atopic disorders in adults with *Helicobacter pylori* infection. Eur. J. Gastroenterol. Hepatol. 15, 637–640.

McDonald, W.I., Compston, A., Edan, G., Goodkin, D., Hartung, H.P., Lublin, F.D., McFarland, H.F., Paty, D.W., Polman, C.H., Reingold, S.C., Sandberg-Wollheim, M., Sibley, W., Thompson, A., van den Noort, S., Weinshenker, B.Y., Wolinsky, J.S., 2001. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. Ann. Neurol. 50, 121–127.

Ponsonby, A.L., van der Mei, I., Dwyer, T., Blizzard, L., Taylor, B., Kemp, A., Simmons, R., Kilpatrick, T., 2005. Exposure to infant siblings during early life and risk of multiple sclerosis. JAMA 26, 463–469.

Stene, L.C., Nafstad, P., 2001. Relation between occurrence of type 1 diabetes and asthma. Lancet 357, 607–608.

Su, J.J., Osoegawa, M., Matsuoka, T., Minohara, M., Tanaka, M., Ishizu, T., Mihara, F., Taniwaki, T., Kira, J., 2006. Upregulation of vascular growth factors in multiple sclerosis: Correlation with MRI findings. J. Neurol. Sci. 243, 21–30.

Wender, M., 2003. Prevalence of *Helicobacter pylori* infection among patients with multiple sclerosis. Neurol. Neurochir. Pol. 37, 45–48.

Yazdanbakhsh, M., Kremsner, P.G., van Ree, R., 2002. Allergy, parasites, and the hygiene hypothesis. Science 296, 490–494.

Please cite this article as: Li, W. et al. *Helicobacter pylori* infection is a potential protective factor against conventional multiple sclerosis in the Japanese population. Journal of Neuroimmunology (2007), doi:10.1016/j.jneuroim.2006.12.010