Journal of Neuroimmunology 305 (2017) 1–4

Contents lists available at ScienceDirect

Journal of Neuroimmunology

journal homepage: www.elsevier.com/locate/jneuroim

Short communication

Memory B cells in Guillain-Barré syndrome

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ARTICLE INFO

Article history:
Received 26 October 2016
Received in revised form 31 December 2016
Accepted 11 January 2017

Keywords:
Guillain-Barré syndrome
memory B cell

ABSTRACT

IgG autoantibodies against gangliosides show the highest titers at the disease onset of axonal Guillain-Barré syndrome (GBS), in which there are no IgM anti-ganglioside antibodies. We hypothesized that memory B cells take part in the development of producing IgG autoantibodies. In this study, we analyzed the memory B cells in patients with GBS using flow cytometry. There was significantly higher percentage of memory B cells in patients with GBS than the healthy controls. The Spearman correlation analysis demonstrated that increased percentage of memory B cells was positively correlated with the clinical severity of the patients with GBS. Our study provides the evidences that memory B cells may be involved in mechanism of GBS.

1. Introduction

Antecedent infectious events trigger the production of autoantibodies attacking the peripheral nerve and result in the onset of Guillain-Barré syndrome (GBS) (Yuki and Hartung, 2012). The first exposure to infectious agents usually activates the B cell to differentiate into IgM-secreting plasma cells. And then, the host immune will keep the memory of previous infections by generating memory B cells in germinal center (Perez et al., 2014). Memory B cells are distinguished from other B cell subsets by an increased lifespan, faster and stronger response to infectious stimulation. Following the second challenge to the initial infectious agent, memory B cells will rapidly proliferate and take the isotype class switch to produce the IgG-predominant antibody (Liu et al., 1996). The histopathological features divide the GBS into demyelinating and axonal subtype, acute inflammatory demyelinating polyneuropathy (AIDP) and acute motor axonal neuropathy (AMAN). In AMAN, the IgG type of antibodies against the gangliosides have been frequently presented during the onset of this disease (Koga et al., 2003; Odaka et al., 2001; Yuki et al., 1995), which suggested that memory B cells may be involved in the pathogenesis of this disease. In this study, we demonstrated that memory B cells increased in peripheral blood of patients with GBS and were positively correlated with the clinical severity of this disease.

2. Materials and methods

2.1. Patients and blood samples

A total of 27 patients fulfilled with the criteria of GBS (Wakerley et al., 2014) and 36 matched healthy donors were recruited into this study. Our study received prior approval by the Ethics Committee of Affiliated Hospital of Jining Medical University and written consent from each donor was obtained. The severity of GBS was evaluated as previously described (van der Meche and Schmitz, 1992). At the acute phase (within 2 weeks after onset and before treatment), early recovery phase (4 weeks after onset) and late recovery phase (8 weeks after onset), peripheral venous blood was collected from each donor. Anti-gangliosides IgG antibodies were detected by an enzyme-linked immunosorbent assay as previously described (Yuki et al., 1997). According to the results, the patients were divided into anti-gangliosides IgG positive group (n = 6) and negative group (n = 21). According to the negative or positive antecedent infection history, the patients were divided into antecedent infection positive (n = 8) and negative group (n = 17). According to the treatment, the patients were divided into intravenous

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http://dx.doi.org/10.1016/j.jneuroim.2017.01.004
0165-5728/

immunoglobulin (IVIG) (n = 10) and plasma exchange (PE) group (n = 11). The details of patients with GBS were shown in Table 1.

2.2. Flow cytometry

Anti-human CD19-FITC mAb (FITC mouse IgG1κ isotype control) (Miltenyi Biotec, Bergisch Gladbach, Germany), anti-human CD27-PE mAb (PE mouse IgG1κ isotype control) (Miltenyi Biotec) were used to stain the memory B cells. In brief, 200 μl of whole blood were incubated with the indicated antibodies in the darkness at room temperature for 15 min. After erythrocytes lysis, the cells were washed using 0.1 M phosphate buffer solution and then submitted to a FACScalibur (BD Biosciences, San Jose, CA). Data for 5×10^4 cells/sample were acquired and analyzed using CellQuest software (BD Biosciences).

2.3. Statistical analysis

The data were shown as mean ± standard error. Independent samples *t*-test was used to compare the percentage of memory B cells between patients with GBS and healthy donors. One-Way ANOVA test was used to compare the percentage of memory B cells in patients with GBS among different phases. Mann-Whitney *U* test was used to compare the changes of percentage of memory B cells before and after treatment in patients with GBS between different groups. The relationship between the percentage of memory B cells in the peripheral blood of patients with GBS and the Hughes' disability score was analyzed by Spearman's correlation. Analysis was performed with the SPSS 19.0 analysis software, and a *p*-value of 0.05 was considered significant.

3. Results

The percentage of memory B cells in patients with GBS at the acute phase (0.96 ± 0.18) was significantly higher than those in the healthy controls (0.43 ± 0.05) ($p < 0.001$). Along with the process of the disease, percentage of memory B cells in patients with GBS gradually decreased at early and late recovery phase (0.72 ± 0.06 and 0.51 ± 0.05, respectively). There was significantly lower percentage of memory B cells at the late recovery phase than acute phase in patients with GBS ($p < 0.001$). There was no significant difference in the percentage of memory B cells in GBS patients between the acute phase and the early recovery phase ($p = 0.06$). The percentage of memory B cells in patients with GBS at the acute phase was positively correlated with the Hughes' disability score ($p = 0.01$, r = 0.82) (Fig. 1). There was no difference in the percentage of memory B cells between anti-gangliosides IgG positive group and negative group ($p = 0.7$). There was no difference in the percentage of memory B cells between antecedent infection positive group and the negative group ($p = 0.9$). There was no difference in changes of percentage of memory B cells before and after treatment in patients with GBS between IVIG and PE group ($p = 0.17$, between onset and early recovery phase; $p = 0.43$, between onset and late recovery phase).

4. Discussion

In humans, expression of CD27 distinguishes the memory B cells ($CD27^+$) from those naive B cells ($CD27^-$) (Agematsu et al., 1997; Tangye et al., 1998). It had been reported that memory B cells play critical roles in multiple autoimmune diseases, such as systemic lupus erythematosus and rheumatoid arthritis (Odendahl et al., 2000; Sellam et al., 2011). We hypothesized that antecedent infections activate memory B cells to secrete the IgG autoantibodies may contribute to the pathogenesis of GBS. In this study, our results demonstrated that memory B cells may participate in mechanism of GBS.

We found that $CD19^+CD27^+$ memory B cells significantly increased during the acute phase of GBS and reduced along with the recovery of this disease. Further analysis of Spearman's correlation showed a positive correlation between clinical severity of GBS and the percentage of memory B cells in peripheral blood of patients, which supports that memory B cells participates in the mechanism of GBS. The intervals between antecedent infection and the neuropathy onset were about 1–2 weeks for respiratory infections and 1 week or less for gastrointestinal infections (Yuki, 2001). Given that it usually takes more than one week for the host to produce IgG antibodies after the first exposure to infectious agents, we hypothesized that increased memory B cells in patients with GBS may explain why the intervals between antecedent infection and the onset of GBS in parts of patients were less than one week. To

Table 1
Demographic and clinical features of patients with Guillain-Barré syndrome.

Number	Sex/age	Antecedent events	Hughes' score at entry	NCS	IgG antibodies	Treatment
1	F/59	URTI	2	Demyelinating	–	PE
2	M/51	–	4	Demyelinating	–	IVIG
3	F/23	URTI	3	Equivocal	–	IVIG
4	F/16	–	2	Demyelinating	–	IVIG
5	M/15	–	2	Axonal	–	IVIG
6	M/44	–	1	Demyelinating	GT1a, GM1, GQ1b	ST
7	M/50	–	4	Equivocal	GT1a	IVIG
8	F/72	–	2	Demyelinating	GM1	IVIG
9	M/33	URTI	0	Equivocal	–	ST
10	F/52	Diarrhea	3	Demyelinating	–	IVIG
11	F/57	–	4	Demyelinating	–	IVIG
12	M/23	Diarrhea	3	Equivocal	–	PE
13	M/48	URTI	4	Demyelinating	–	IVIG
14	F/70	–	3	Equivocal	–	PE
15	M/52	Diarrhea	2	Demyelinating	–	PE
16	F/64	–	2	Demyelinating	–	PE
17	F/42	Spine surgery	1	Equivocal	GM1	PE
18	M/64	–	3	Demyelinating	GM1	PE
19	M/55	–	1	Demyelinating	–	ST
20	F/49	–	3	Demyelinating	–	PE
21	M/74	Spine surgery	4	Axonal	–	PE
22	F/70	–	2	Demyelinating	–	ST
23	M/36	URTI	1	Axonal	–	ST
24	F/37	–	2	Demyelinating	–	PE
25	M/54	–	2	Axonal	–	IVIG
26	F/38	–	5	Demyelinating	–	PE
27	M/44	–	4	Demyelinating	GQ1b, GD1b	PE

URTI, upper respiratory tract infection; NCS, nerve conduction study; PE, plasma exchange; IVIG, intravenous immunoglobulin; ST, supportive treatment.