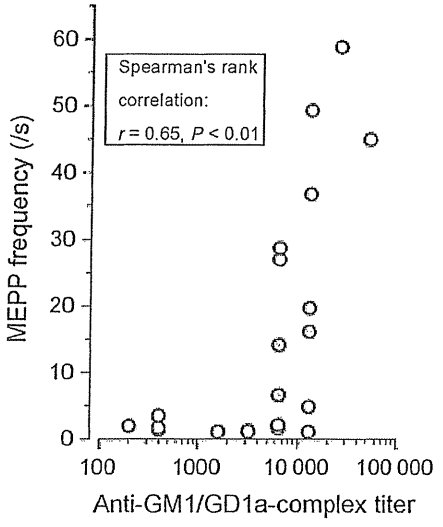

Figure 4 Positive correlation between anti-GM1/GD1a-complex titer of sera and the miniature end-plate potential (MEPP) frequency induced at mouse neuromuscular junctions (NMJs). Plot graph of the average MEPP frequency at NMJs of diaphragm muscles from GD3-synthase knockout mice induced by anti-GM1/GD1a-complex-positive GBS sera against the titer of the anti-GM1/GD1a-complex antibodies. Spearman's rank correlation test showed a highly significant positive correlation ($r = 0.65$, $P < 0.01$).

in the mouse diaphragm/phrenic nerve *ex vivo* experimental model. Roughly half of the 31 anti-ganglioside-complex sera tested in the current experiments induced the effects, which were similar to those observed earlier with antibodies and sera with activity against either single gangliosides GQ1b, GD1a, GM1 or GD1b.[4,11,12,21] In the set of 21 anti-GM1/GD1a-complex positive sera, we found a statistically highly significant correlation between the MEPP frequency elevation observed in the electrophysiological experiments and the titer of this specific anticomplex antibody in the sera and, furthermore, an association with complement activation as quantified in fluorescence microscopical analyses. In previous studies using sera or antibodies against single gangliosides, we showed that the utmost consequence at the mouse NMJ is a block of evoked ACh release as a result of presynaptic focal complement-mediated lysis, leading to muscle paralysis.[4] Although we did not structurally monitor in the present studies whether or not anti-ganglioside complex sera induced these end-point effects, some of them certainly caused a (partial) block of the diaphragm muscle contraction evoked by nerve stimulation, as judged visually. However, especially with the sera that only induced moderate increases in MEPP frequency, it is to be expected that they would not, or only after periods much longer than the current observation period of 1 h, lead to transmission block.

Thus, we here for the first time show that anti-ganglioside-complex antibodies are capable of binding to living neuronal membranes and, by activating complement, can induce pathophysiological effects. These antibodies are therefore likely to be of pathogenic relevance, as also suggested by the clinical association with specific patterns of paralysis and, in some patient groups, mechanical ventilation.[2,8] In a previous study, anti-GM1/GD1a-complex serum positivity was associated with a pure motor variant of GBS without severe cranial nerve involvement,[9] suggesting a specific effect of these antibodies on motor axons. Our finding of deleterious effects of anti-ganglioside-complex antibodies at mouse motor nerve terminals suggests that these antibodies might, apart from causing motor axonal dysfunction, induce some degree of NMJ synaptopathy in GBS patients, potentially contributing to the paralytic symptoms.

As a result of the limited availability of most of the patient sera for repetitive experimental study and because of the heterogeneity of the anti-ganglioside(-complex) characteristics of the studied sera, some complexities of our results remain unresolved. First, not all sera induced the deleterious effects at mouse NMJs. Second, some of the active sera, especially those from the anti-GM1/GQ1b-complex positive series, caused only moderate effects; that is, the MEPP frequency remained lower than 10/s, as compared with values of >20 MEPP/s induced by the positive control mAbs and many of the active anti-GM1/GD1a-complex sera. These differences might relate to the titer and affinity variations of anti-ganglioside-complex antibodies amongst sera, together with the likely existence of an antibody binding threshold for the induction of pathophysiological effects at NMJs. Indeed, pathophysiological inactive or less active sera generally had low anti-ganglioside-complex titers and, at least in the anti-GM1/GD1a-complex series, statistical analysis showed a clear correlation between the elevated MEPP frequency and antibody titer. Third, many active sera, especially the anti-GM1/GD1a-complex positive sera, contained additional activities against single gangliosides GM1 and/or GD1a (Table 1), which in principle might have contributed to the effects. However, these single ganglioside antibody titers were generally (very) low, both in absolute sense, as well as relative to the titers of the anti-GM1/GD1a-complex antibody in these sera. Furthermore, there was no statistically significant correlation between the titer

of anti-GM1 or anti-GD1a antibodies and the elevated MEPP frequency observed with the sera. Still, this copresence of anti-single-ganglioside antibodies complicates interpretation, in particular, because four anti-GM1/GD1a-complex sera without additional activity against GM1 or GD1a lacked effects. This could be as a result of their only low-positive anti-GM1/GD1a-complex titers, but this might also suggest that besides anti-GM1/GD1a-complex activity, some additional anti-glycolipid or anti-glycolipid-complex activity is required for the neuropathophysiological effects. Any potential copresence of anti-GQ1b single-ganglioside antibody in the anti-GM1/GD1a-complex sera active at GD3s-KO NMJs could not have been of influence, because GQ1b ganglioside is not expressed in the plasma membranes of GD3s-KO mice (Fig. 1). The strongest direct evidence for a neuropathophysiological effect of anti-GM1/GD1a-complex antibody came from the study of serum 5. The anti-GM1/GD1a-complex antibodies in this serum unambiguously were solely responsible for the complement-mediated neuropathophysiological effects at GD3s-KO NMJs, because this serum contained no additional anti-GD1a or -GM1 single-ganglioside antibodies. Some activity against GD1b ganglioside and GD1b- and GQ1b-containing ganglioside complexes was detected in this serum (data not shown), but this was irrelevant because GD3s-KO tissue lacks the b-series gangliosides GD1b and GQ1b (Fig. 1). Thus, the results obtained with this particular serum clearly provide proof-of-principle that GBS anti-ganglioside-complex antibodies can induce neuropathophysiological effects at living neuronal membranes.

Activity against the single gangliosides GM1 or GQ1b was less of a confounding factor in the GM1/GQ1b-complex group, where seven of the 10 sera lacked single ganglioside antibodies. However, just three of those induced MEPP frequency elevations at wild-type NMJs and these effects were only rather modest in magnitude. In addition, complement deposition and muscle fibre twitching did not correlate very well with elevation of MEPP frequency. No statistically significant correlation was found between the anti-GM1/GQ1b-complex antibody titer of the sera in this series and the MEPP frequency that was observed at wild-type NMJs. This shows that anti-GM1/GQ1b antibodies generally only induce relatively weak neuropathogenic effects in this experimental model.

Anti-GM1/GD1a-complex sera induced either no neuropathophysiological effects or much less intense effects at wild-type NMJs, as compared with GD3s-KO NMJs. This shows the requirement of an elevated anti-GM1/GD1a-complex antigen density for anti-GM1/GD1a-complex antibodies to become neuropathogenic, because the a-series gangliosides GM1 and GD1a are upregulated in neuronal membranes of GD3s-KO mice, including motor nerve terminals.[12,16] The ganglioside and ganglioside-complex expression pattern in different peripheral nerve membrane domains might vary considerably, both within and between species. It is even possible that certain GBS patients express particular predisposing ganglioside-complex densities or configurations. Development of high-affinity mouse mAbs specific for ganglioside-complexes will be an essential next step, allowing more detailed experimental studies in which these issues can be explored.

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MINI-REVIEW

Antibodies against ganglioside complexes in Guillain–Barré syndrome and related disorders

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Abstract

Guillain-Barré syndrome (GBS) is acute autoimmune neuropathy, often subsequent to an infection. Serum anti-ganglioside antibodies are frequently elevated in titer. Those antibodies are useful diagnostic markers and possible pathogenetic factors. Recent data demonstrated that sera from some patients with GBS react with ganglioside complexes (GSCs) consisting of two different gangliosides, but not with each constituent ganglioside. Those antibodies may specifically recognize a new conformational epitope formed by two gangliosides. In particular, the antibodies against GD1a/GD1b and/or GD1b/GT1b complexes are associated with severe GBS requiring artificial ventilation. The antibodies to GM1/GalNAc–GD1a and those to GSCs containing GQ1b or GT1a are associated with pure motor GBS and Fisher syndrome, respectively. In contrast, the binding activities of the antibodies highly specific to GD1b are strongly inhibited by the addition of GD1a to GD1b. Gangliosides along with other components as cholesterol are known to form lipid rafts, in which two different gangliosides may form a new conformational epitope. Future investigation is necessary to elucidate the roles of GSCs in the plasma membrane and of the clinical relevance of the anti-GSCs antibodies.

Keywords: ganglioside, Guillain-Barré syndrome, membrane microdomain, peripheral nerve.

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Anti-ganglioside antibodies, mostly IgG type, are present in the sera from approximately 60% of patients with Guillain-Barré syndrome (GBS), acute immune-mediated polyradiculoneuropathy (Willison and Yuki 2002; Kusunoki *et al.* 2008; Van Doorn *et al.* 2008). Because the presence of anti-ganglioside antibodies in the acute-phase sera is a characteristic feature of GBS, those antibodies can be used as diagnostic markers of GBS. There are many molecular species of gangliosides, named depending on the carbohydrate sequences. Each ganglioside has unique distribution within the PNS. Considering the gangliosides are localized in the plasma membrane with their carbohydrate portions extended to the extracellular spaces, the anti-ganglioside antibodies may function in the pathogenesis of GBS through antibody-antigen interaction in PNS.

IgG anti-GQ1b antibody is one of the best studied antibodies. Ig anti-GQ1b antibodies are specifically associated with a variant of GBS, Fisher syndrome (FS) characterized by ophthalmoplegia and ataxia (Chiba *et al.* 1992). Anti-GQ1b monoclonal antibody specifically immunostains paranodal myelin of human cranial nerves innervating extraocular muscles (Chiba *et al.* 1993) and some large neurons in dorsal root ganglia (Kusunoki *et al.* 1999). It has recently been reported that the neuromuscular junctions of human extraocular muscles are richly bound by the antibodies against GQ1b and GT1a (Liu *et al.* 2009). Thus, the anti-GQ1b antibodies may cause opthalmoplegia and ataxia by binding to the regions where GQ1b is densely localized.

Measurement of anti-ganglioside antibodies has been conducted with ELISA or TLC-immunostaining by the use of purified single ganglioside antigens. Gagliosides have characteristics of forming clusters in the plasma membrane

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Abbreviations used: AMCBN, acute motor conduction block neuropathy; FS, Fisher syndrome; GBS, Guillain-Barré syndrome; GSC, ganglioside complex; LOS, lipooligosaccharides.

(Hakomori 2002). In the clusters, the carbohydrate structure of a ganglioside may interact with each other to form a novel epitope. We recently demonstrated that some GBS patients had serum antibodies that specifically recognize the novel glycoepitopes formed by two individual ganglioside molecules and named such antibodies as 'anti-ganglioside complex (GSC) antibodies' (Kaida *et al.* 2004).

Antibodies to ganglioside complexes in GBS

Antibodies to GD1a/GD1b and GD1b/GT1b complexes in severe GBS

Anti-GD1a/GD1b complex antibodies are the first identified antibodies against GSCs. We investigated a serum from a GBS patient who showed acute severe flaccid tetraparesis and needed artificial ventilation. We found an unidentified immuno-reactive band in the position just below GD1a on TLC of a crude ganglioside fraction from bovine brain. The serum was not reactive with any of such purified gangliosides as GM1, GM2, GM3, GD1a, GD1b, GD3, GalNAc–GD1a, GT1b, and GQ1b. But the serum IgG bound strongly to the well coated with the mixture of GD1a and GD1b gangliosides (GD1a/GD1b complex). When GD1a and GD1b were developed in the same lane on TLC using a developing solvent, chloroform/methanol/0.2%$CaCl_2 \cdot 2H_2O$ (50 : 45 : 10), the serum IgG strongly immunostained just the overlapping portion between GD1a and GD1b. When another developing solvent (C/M/0.2%$CaCl_2 \cdot 2H_2O$, 30/65/10) that completely separated the positions of GD1a and GD1b was used, no immunoreaction was identified. Those data indicate that mixing GD1a and GD1b may produce a new conformational glycoepitope which is different from that of GD1a or GD1b alone and the antibody in sera from the above patient may specifically recognize such a new glycoepitope.

We next investigated antibodies in sera from 234 GBS patients with ELISA using a mixture of two of the four major gangliosides (GM1, GD1a, GD1b and GT1b) (Kaida *et al.* 2007). The sera with anti-GSC antibodies often exhibited to some extent reactivity with constituent gangliosides of the GSCs. When optical density for the anti-GD1a/GD1b antibody was 0.2 higher than that corresponding to anti-GD1a or anti-GD1b antibody or it was more than the sum of those of anti-GD1a and anti-GD1b antibodies, the sera were judged to be anti-GD1a/GD1b-positive. The same criteria also were applied to the other GSCs. The cutoff value (0.2) for anti-GSC antibodies was decided arbitrarily. The results showed that 39 of 234 patients (17%) had antibodies against at least one of the mixture antigens. All the 39 patients had anti-GM1/GD1a antibodies, 27 had anti-GM1/GT1b antibodies, 16 had anti-GD1a/GD1b antibodies, and 13 had GD1b/GT1b antibodies. Most of anti-GD1a/GD1b or anti-GD1b/GT1b antibody reacted also with GM1/GT1b as well as GM1/GD1a. Immunoabsorption study suggested that anti-GSC antibodies specifically react with clustered glycoepitopes common to these GSCs, rather than individually with each GSC. An epitope formed by a combination of [Galβ1-3GalNAc] and [NeuAcα2-3Galβ1-3GalNAc] in the terminal moieties of ganglio-*N*-tetraose structures is likely to be essential for the antibody binding. Among them, antibodies against GD1a/GD1b and GD1b/GT1b complexes were significantly associated with severe GBS requiring artificial ventilation (Kaida *et al.* 2007). Those antibodies can be useful markers of severe GBS. Future study is needed to clarify why anti-GD1a/GD1b and GD1b/GT1b antibodies are associated with severe disabilities.

Antibodies to ganglioside complexes including GQ1b

Because FS is considered to be a variant of GBS, we extended an investigation of anti-GSC antibodies to FS patients. Presence of anti-ganglioside complexes antibodies in FS therefore was investigated with ELISA using seven ganglioside antigens; GM1, GM2, GD1a, GD1b, GT1a, GT1b and GQ1b (Kaida *et al.* 2006).

Acute phase serum samples were collected from 12 FS patients, 10 of whom had IgG anti-GQ1b antibodies. ELISA results showed that seven patients had antibodies to GSCs such as GQ1b/GM1, GQ1b/GD1b, GQ1b/GD1a, GQ1b/GT1b, GT1a/GM1, GT1a/GD1b, and GT1a/GD1a, but not to the complexes without GQ1b and GT1a. One patient had no anti-GQ1b or anti-GT1a antibodies, but had antibodies to GQ1b/GM1 and GT1a/GM1. Specific immunoreactivities against the overlapping portion of the two gangliosides were confirmed by TLC-immunostaining. In contrast to GBS, no FS patients had antibodies to the complexes consisting of two of the four major gangliosides, GM1, GD1a, GD1b and GT1b.

The results of anti-GSCs antibody assay on larger number of patients with FS and those with GBS with opthalmoplegia indicated that the serum antibodies could be subdivided into the three groups (Kanzaki *et al.* 2008): (i) antibodies specific to GQ1b and/or GT1a without anti-GSCs reactivity; (ii) antibodies that recognize a combination of [Galβ1-3GalNAc] and [NeuAcα2-8 NeuAcα2-3Galβ1-3GalNAc] in the terminal residues of ganglio-*N*-tetraose structures, such as antibodies to GQ1b/GM1, GQ1b/GD1b, GT1a/GM1, GT1a/GD1b (Fig. 1); and (iii) antibodies that recognize a combination of [NeuAcα2-3Galβ1-3GalNAc] and [NeuAcα2-8 NeuAcα2-3Galβ1-3GalNAc] in the terminal residues, such as antibodies to GQ1b/GD1a, GT1a/GD1a, GQ1b/GT1b, GT1a /GT1b. In addition, recent report showed that some patients have the antibodies specific to GQ1b/GA1 (Ogawa *et al.* 2009).

Sensory signs were infrequent in FS patients with antibodies to GQ1b/GM1 but were frequent in patients with other types of antibodies. However, the clinical relevance of such anti-GSC antibodies needs to be investigated in future.

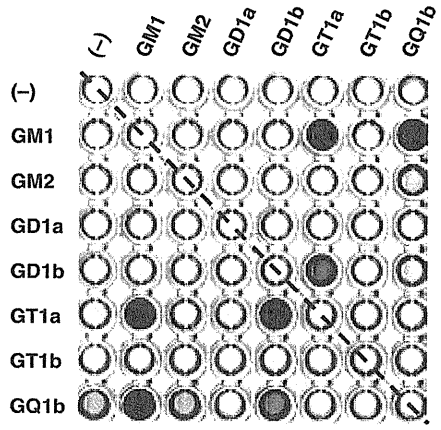

Fig. 1 An ELISA plate showing the binding activities of a serum antibody that recognizes a combination of [Galβ1-3GalNAc] and [NeuAcα2-8NeuAcα2-3 Galβ1-3GalNAc] in the terminal residues. All the wells in each line and column were coated with a respective ganglioside (e.g. the wells in the first line and column were coated only with a single ganglioside, the well in the eighth line and the second column was coated with GQ1b and GM1), except for those on the oblique dotted line that were uncoated control wells. The antibody binds strongly to GQ1b/GM1, GQ1b/GD1b, GT1a/GM1 and GT1a/GD1b but only weakly to GQ1b.

Antibodies to GM1/GalNAc–GD1a complex in pure motor GBS

IgG antibodies against GM1 or those against GalNAc–GD1a are known to closely correlate with acute motor axonal neuropathy (Kaida *et al.* 2000; Willison and Yuki 2002). We investigated antibody activities against the mixture of GM1 and GalNAc–GD1a (GM1/GalNAc–GD1a complex) in a large population of patients with GBS. The results showed that ten of 224 GBS patients had IgG antibodies to the GM1/GalNAc–GD1a complex (Kaida *et al.* 2008a).

We then analyzed the clinical and electrophysiologic findings of those 10 anti-GM1/GalNAc–GD1a-positive patients. Respiratory infections preceded the neurological onset in six cases and gastrointestinal infections in two cases. Therefore, although *Campylobacter jejuni* is an infectious agent that frequently causes the antecedent infection of GBS cases with anti-GM1 and anti-GalNAc–GD1a antibodies, *C. jejuni* may not be the major infectious agent inducing anti-GM1/GalNAc–GD1a complex antibodies. Cranial nerve involvement and sensory signs are infrequent. Early motor conduction block at intermediate nerve segments was found in five patients. Generally, the response to therapy was good. According to the criteria established by Hadden *et al.* (1998), four were categorized as demyelinative and two were axonal. When judged by other criteria (Ho *et al.* 1995), four were demyelinative and three were axonal.

Table 1 Representative anti-GSCs antibodies in GBS and FS

Antigen	Associated disease	Frequency (%)	Clinical features
GD1a/GD1b	GBS	7	Severe GBS
GD1b/GT1b	GBS	6	Severe GBS
GM1/GalNAc–GD1a	GBS	4	Pure motor GBS AMCBN
GQ1b/GM1 and related GSCs	FS	41	Infrequent sensory dysfunction
	GBS with OP	28	
GQ1b/GD1a and related GSCs	FS	6	
	GBS with OP	19	

GSC, ganglioside complex; GBS, Guillain-Barré syndrome; FS, Fisher syndrome; AMCBN, acute motor conduction block neuropathy; OP, ophthalmoplegia.
GQ1b/GM1 and related GSCs, GQ1b/GM1, GQ1b/GD1b, GT1a/GM1, GT1a/GD1b; GQ1b/GD1a and related GSCs, GQ1b/GD1a, GT1a/GD1a, GQ1b/GT1b, GT1a /GT1b.

The clinical findings of the 10 GBS patients were consistent with a pure motor variant of GBS. Clinical features of anti-GM1/GalNAc–GD1a IgG-positive GBS resemble those of acute motor conduction block neuropathy (AMCBN), in view of preserved sensory function, early conduction block at intermediate nerve segments and good recovery (Capasso *et al.* 2003). IgG anti-GM1 antibody (and sometimes anti-GalNAc–GD1a antibody) was reported in their sera. However, IgG anti-GM1 or anti-GalNAc–GD1a antibodies are frequently detected in sera of acute motor axonal neuropathy type GBS and conduction block is not common in such cases. Anti-GM1/GalNAc–GD1a antibody is likely to cause early reversible changes on the axolemma and may be more closely associated with AMCBN than the anti-GM1 or anti-GalNAc–GD1a antibody. GM1 and GalNAc–GD1a may form a complex in the axolemma at nodes of Ranvier or paranodes of the motor nerves, and may be a target antigen in pure motor GBS; especially in the form of AMCBN.

Representative anti-GSCs antibodies in GBS and FS are listed in the Table 1.

Antibodies against ganglioside complexes in chronic neuropathies

Nobile-Orazio *et al.* (2010) investigated serum IgM antibodies to GSCs in such chronic neuropathies as multifocal motor neuropathy, chronic inflammatory demyelinating polyradiculoneuropathy and IgM paraproteinemic neuropathy. As a result, one of 34 chronic inflammatory demyelinating polyradiculoneuropathy patients had IgM antibody activity to GT1b/GM1 and GT1b/GM2, and one of 23 IgM paraproteinemic neuropathy patients had IgM anti-GM2/GD1b activity.

Production of antibodies against ganglioside complexes

In GBS and related disorders subsequent to *C. jejuni* infection, anti-ganglioside antibodies are shown to be induced by the immune reaction against lipo-oligosaccharides (LOS) of pathogens causing antecedent infection (Willison and Yuki 2002; Van Doorn *et al.* 2008). A similar mechanism can be speculated in the production of anti-GSC antibodies. Kuijf *et al.* (2007) recently reported that such anti-GSC antibodies as anti-GM1/GD1a and GQ1b/GD1a cross-reacted to LOS from the autologous *C. jejuni* strain, indirectly demonstrating that the LOS contained GSC-like structures. However, carbohydrate structures expressed in the LOS may not exactly be the combination of the two carbohydrate chains expected from the reactivity of the serum anti-GSC antibodies.

Inhibition of the reactivity of the anti-ganglioside antibody by another coexistent ganglioside

If the interaction of two gangliosides creates a new epitope with conformational changes, the binding acitivity of the antibody highly specific to one ganglioside may be lessened by the addition of another ganglioside to make an antigen mixture.

We investigated sera from 17 GBS patients who had IgG antibody reactive only with GD1b in routine antibody assay. For those sera, antibody activity against a mixture of GD1b and another ganglioside was examined and compared the activity with that against GD1b alone. The results showed that the addition of GD1a, GT1a, GT1b, GQ1b and GalNAc–GD1a to GD1b caused marked decrease of the binding activity of anti-GD1b antibodies, suggesting that those gangliosides may interact with GD1b to make a novel epitope which cannot be easily recognized by the anti-GD1b antibodies (Kaida *et al.* 2008b).

In addition, the reduction rates of the binding activities caused by the addition of such gangliosides as GD1a, GT1b, GQ1b and GalNAc–GD1a were significantly more in the antibodies from ataxic patients than in those from non-ataxic patients. The addition of another ganglioside may cause conformational change. Therefore, the more specific the antibody is, the more affected its reactivity should be. It therefore suggests that the anti-GD1b IgG antibodies in ataxic patients may be more specific to GD1b than those in patients without ataxia. This may provide further evidence to the association between anti-GD1b antibody and ataxia (Kusunoki *et al.* 1996).

Thus, the antibodies specific to GD1a/GD1b complex are associated with severe GBS (Fig. 2) and those specific to GD1b itself are associated with the development of ataxia (Fig. 3).

A similar inhibitory effect of neighboring gangliosides has recently been reported in the case of anti-GM1 antibodies by

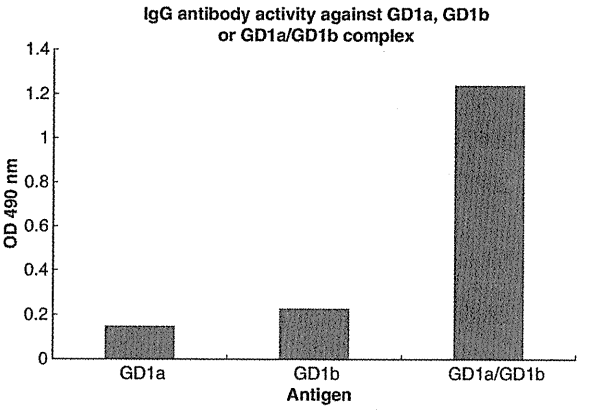

Fig. 2 ELISA result on a serum sample from a patient with severe Guillain-Barré syndrome (Kaida *et al.* 2004). This patient's serum IgG shows strong reaction with a mixture of GD1a and GD1b (GD1a/GD1b) but reacts only weakly with GD1a or GD1b alone.

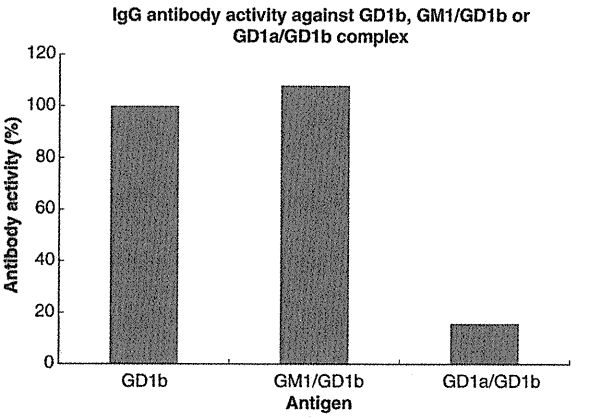

Fig. 3 The IgG antibody activities to mixture antigens in sera from nine GBS patients with ataxia who had only IgG anti-GD1b antibody in routine antibody assay (Kaida *et al.* 2008b). Bars of GM1/GD1b and GD1a/GD1b showed the average activities of the nine patients. Compared with the antibody activity to GD1b alone (100%), the activity was markedly reduced because of the addition of GD1a to GD1b antigen whereas the addition of GM1 did not affect the antibody activity.

Greenshields *et al.* (2009). Negative effects by gangalioside complexes on the binding of IgM anti-GM1 antibodies in sera from patients with chronic immune-mediated neuropathies, particularly multifocal motor neuropathy, have also been reported (Nobile-Orazio *et al.* 2010).

Future studies on the anti-GSC antibodies in the pathogenesis of autoimmune neuropathies

Gangliosides are located in the cell membranes with carbohydrate portions on the outer surfaces, and are preferentially

packaged with cholesterol, forming lipid rafts. Within rafts, gangliosides are considered to interact with important transmembrane receptors or signal transducers (Simons and Ikonen 1997; Hakomori 2002). Anti-GSC antibodies may cause dysfunction of the axon or Schwann cells through their binding to clustered epitopes of glycosphingolipids in the plasma membrane microdomains. Future study on the localization of each ganglioside complex is needed. Animal model of the autoimmune neuropathy mediated by anti-GSC antibodies should also be developed. Such investigations may lead to the understanding of the roles of GSCs in the plasma membrane and of the clinical relevance of the anti-GSCs antibodies.

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Interleukin 6 signaling promotes anti-aquaporin 4 autoantibody production from plasmablasts in neuromyelitis optica

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Neuromyelitis optica (NMO) is an inflammatory disease affecting the optic nerve and spinal cord, in which autoantibodies against aquaporin 4 (AQP4) water channel protein probably play a pathogenic role. Here we show that a B-cell subpopulation, exhibiting the $CD19^{int}CD27^{high}CD38^{high}CD180^{-}$ phenotype, is selectively increased in the peripheral blood of NMO patients and that anti-AQP4 antibodies (AQP4-Abs) are mainly produced by these cells in the blood of these patients. These B cells showed the morphological as well as the phenotypical characteristics of plasmablasts (PB) and were further expanded during NMO relapse. We also demonstrate that interleukin 6 (IL-6), shown to be increased in NMO, enhanced the survival of PB as well as their AQP4-Ab secretion, whereas the blockade of IL-6 receptor (IL-6R) signaling by anti–IL-6R antibody reduced the survival of PB in vitro. These results indicate that the IL-6–dependent B-cell subpopulation is involved in the pathogenesis of NMO, thereby providing a therapeutic strategy for targeting IL-6R signaling.

neuroinflamatory disease | autoimmunity | multiple sclerosis | central nervous system | IL-6 receptor blockade

Neuromyelitis optica (NMO) is an inflammatory demyelinating disorder characterized by recurrent attacks of severe optic neuritis and myelitis. Unlike the conventional form of multiple sclerosis (CMS), the lesions of NMO tend to spare the cerebral white matter, especially during the early stage (1), and even a single episode of attack can cause serious neurological deficits such as total blindness and paraplegia. Accordingly, accumulation of irreversible damage to the central nervous system (CNS) along with rapid progression of disability is more frequently found in NMO compared with CMS (2).

NMO can be distinguished from CMS by clinical, neuroimaging, and serological criteria (3). It is now known that serum anti-aquaporin 4 (AQP4) autoantibodies can be used as a disease marker of NMO (1, 2). AQP4 is the most abundantly expressed water channel protein in the CNS and is highly expressed in the perimicrovessel astrocyte foot processes, glia limitans, and ependyma (4). Emerging clinical and pathological observations suggest that anti-AQP4 antibodies (AQP4-Abs) play a key role in the pathogenesis of NMO. Prior studies have documented a significant correlation of serum AQP4-Ab levels with the therapeutic efficacy of plasma exchange during clinical exacerbations of NMO (2, 5). In the CNS lesions of NMO, reduced expression of AQP4 on astrocytes is evident even during the early stage (6), which is followed by the occurrence of vasculocentric destruction of astrocytes associated with perivascular deposition of complement and IgG (7).

On the other hand, recent studies have suggested that AQP4-Abs alone are incapable of causing the clinical and pathological features of NMO. In fact, Hinson et al. emphasized the role of cellular immunity in combination with AQP4-Abs by showing that the attack severity of NMO was not correlated with serum AQP4-Ab levels (8). It was also demonstrated that direct injection of IgGs derived from NMO patients into the brains of naïve mice did not cause NMO-like lesions, although brain tissue destruction associated with leukocyte infiltration was elicited by coinjecting human complement (9). Other groups have shown that the passive transfer of IgGs from NMO patients to rats challenged with induction of experimental autoimmune encephalomyelitis (EAE) may cause a decrease in the expression of AQP4 in astrocytes along with worsening of clinical EAE (10–12). In contrast, the transfer of IgGs to unimmunized rats did not cause any pathology. These results suggest that induction of AQP4-Ab–mediated pathology in NMO depends on the presence of complement, leukocytes, and T cells.

Although AQP4-Ab–secreting cells are a potential target for therapy, detailed characteristics of AQP4-Ab–producing cells have not been clarified yet. Because some NMO patients have elevated serum anti-nuclear and anti–SS-A/SS-B Abs (1), as found in patients with systemic lupus erythematosus (SLE) or Sjögren syndrome, NMO might share common pathological mechanisms with these autoimmune diseases. Kikuchi et al. previously reported that $CD180^{-}$ B cells are activated B cells capable of producing autoantibodies in SLE (13). CD180 is a member of the leucine-rich repeat family of molecules with homology to Toll-like receptor 4 (14), which is highly expressed by naïve and memory B cells but not by plasma cells (15). Odendahl et al. demonstrated that $CD27^{high}CD38^{+}$ B cells, capable of producing high-affinity IgG (16), are increased in the peripheral blood of SLE patients with some correlation to disease activity (17). Considering the phenotypes of autoantibody-producing cells reported in SLE, we analyzed the expression of CD27, CD38, and CD180 on $CD19^{+}$ B cells in the peripheral blood of NMO patients. We found that $CD27^{high}CD38^{high}CD180^{-}$ B cells were significantly increased in AQP4-Ab seropositive patients diagnosed with NMO or NMO spectrum disorder (1) compared with healthy subjects (HS) or CMS patients. Notably, this B-cell subpopulation was found to be a major source of AQP4-Abs in the peripheral blood of AQP4-Ab seropositive patients and depended on interleukin-6 receptor (IL-6R) signaling for survival.

Author contributions: N.C., T.A., S.M., and T.Y. designed research; N.C. and W.S. performed research; Y.M. and S.M. contributed new reagents/analytic tools; N.C., T.A., T.O., M.O., T.T., and T.Y. analyzed data; T.Y. supervised the work; and N.C., T.A., and T.Y. wrote the paper.

The authors declare no conflict of interest.

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Results

$CD27^{high}CD38^{high}CD180^{-}$ B Cells Were Increased in the Peripheral Blood of NMO Patients. Although AQP4-Abs are identified as IgGs (18), no prior study has focused on proportional changes of B-cell subsets in NMO. We therefore performed multicolor flow cytometric analysis of peripheral blood mononuclear cells (PBMC) derived from patients and controls. After starting the study, we soon noticed a remarkable expansion of a distinct B-cell subset in some patients with NMO. The expanded B cells were identified as a population of $CD27^{high}$, $CD38^{high}$, and $CD180^{-}$, and showed lower expression of CD19 than other B cells (Fig. 1*A*). Notably, this population did not express the B-cell marker CD20 (Fig. S1). First, we collected samples from patients in remission and analyzed the pooled data. We found that the proportion of this subpopulation among $CD19^{+}$ B cells was significantly increased in AQP4-Ab seropositive patients with NMO or NMO spectrum disorder (Fig. 1*B*) compared with HS or CMS patients. There was no significant difference in the proportion of this B-cell subpopulation between those with typical NMO and those with NMO spectrum disorder. Furthermore, the frequency of this B-cell subpopulation was correlated with the serum AQP4-Ab titer (Fig. S2). Comparison of paired samples obtained from the same patients during relapse and in remission showed that the $CD27^{high}CD38^{high}CD180^{-}$ B cells further increased during relapse (Fig. 1*C*). In contrast, the frequencies of $CD27^{-}$ naïve B cells (nB) and $CD27^{+}CD38^{-low}$ memory B cells (mB) were not altered in AQP4-Ab seropositive patients compared with controls (Fig. S3). The large majority of seropositive patients were treated with corticosteroids. However, the frequency of $CD27^{high}CD38^{high}CD180^{-}$ cells among $CD19^{+}$ B cells was not correlated with the daily corticosteroid dose given to patients (Fig. S4). Moreover, the increase in cells in NMO patients was still evident compared with that in CMS patients similarly treated with corticosteroids (Fig. S5). Taken together, the selective increase in $CD27^{high}CD38^{high}CD180^{-}$ B cells in seropositive patients was thought to reflect their role in the pathogenesis of NMO but not to be an effect of the corticosteroid treatment.

Expanded Cells Resemble Early Plasma Cells in Gene Expression and Morphology. To gain insights into the developmental stage of the $CD27^{high}CD38^{high}CD180^{-}$ B cells, we quantified the mRNA expression of B-cell–associated transcription factors in sorted cell populations. Compared with nB and mB, this population showed much higher expression of B-lymphocyte-induced maturation protein 1 (Blimp-1) and IFN regulatory factor 4 (IRF4), which are essential for the regulation of plasma cell differentiation (19, 20) (Fig. 2*A*). In contrast, the expression of paired box gene 5 (PAX5), known to be down-regulated in early plasma cell differentiation (21), was reciprocally reduced in the B-cell subset. This gene expression pattern is very similar to that of plasma cells. However, it was notable that the cells of interest expressed CD19, which is not detected in mature plasma cells. Moreover, only 40% of this population expressed the most reliable plasma cell marker CD138 (22). Morphological analysis also confirmed the similarity of this population to plasma cells: they exhibit eccentric nucleus, perinuclear hof region, and abundant cytoplasm. However, they possess a larger nucleus with a lower extent of chromatin clumping compared with $CD138^{+}$ plasma cells derived from HS (Fig. 2*B*). Notably, the $CD138^{+}$ population among $CD27^{high}CD38^{high}CD180^{-}$ cells in NMO patients was

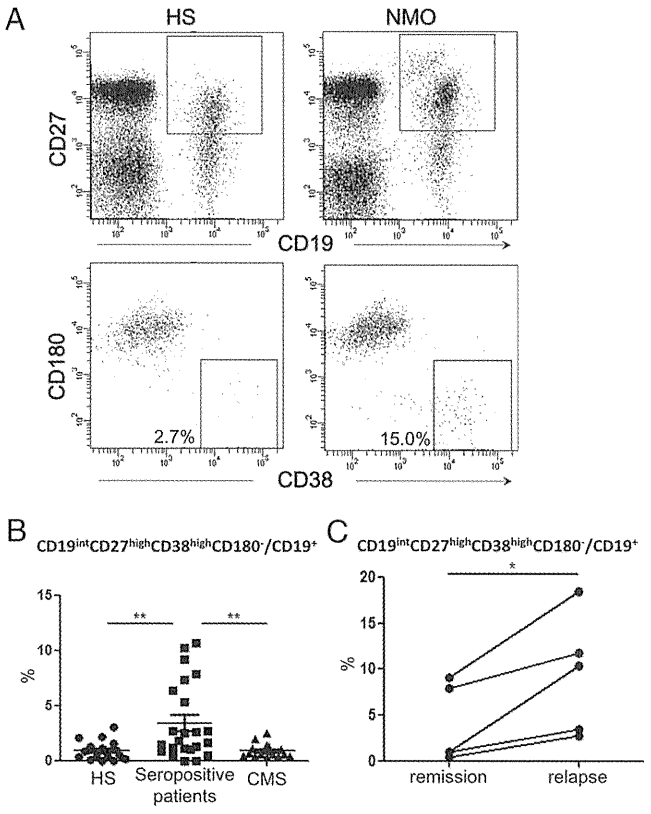

Fig. 1. $CD27^{high}CD38^{high}CD180^{-}$ B cells increased in NMO patients. (*A*) A flow cytometric scheme for the analysis of B-cell subpopulations. PBMC from HS and NMO in remission were stained with fluorescence-conjugated anti-CD19, -CD27, -CD38, and -CD180 mAbs. $CD19^{+}CD27^{+}$ cells were partitioned (*Upper*) and analyzed for expression of CD38 and CD180 (*Lower*). Values represent the percentage of $CD38^{high}CD180^{-}$ cells within $CD19^{+}CD27^{+}$ cells. (*B*) Analysis of the pooled data derived from patients in clinical remission. This shows the percentages of $CD27^{high}CD38^{high}CD180^{-}$ cells within $CD19^{+}$ cells from HS, seropositive patients, and CMS patients (**$P < 0.01$; Tukey's post hoc test). (*C*) Comparison of remission and relapse of NMO. Data obtained from the same patients are connected with lines (*$P < 0.05$; Wilcoxon signed rank test).

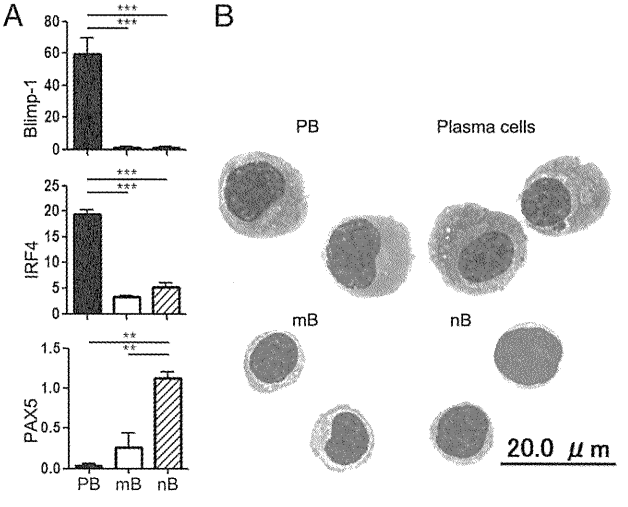

Fig. 2. Resemblance of $CD19^{int}CD27^{high}CD38^{high}CD180^{-}$ cells to plasma cells. (*A*) mRNA expression of Blimp-1, IRF4, and PAX5. B-cell subpopulations [$CD27^{high}CD38^{high}CD180^{-}$ (PB), $CD27^{-}$ naïve (nB), $CD27^{+}CD38^{-low}$ memory (mB)] were sorted by FACS and total RNA was extracted for qRT-PCR analysis. RNA levels were normalized to ACTB for each sample (**$P < 0.01$; ***$P < 0.001$; Tukey's post hoc test). (*B*) May–Grünwald–Giemsa staining of B-cell subpopulations. PB (*Upper Left*), mB (*Lower Left*), and nB (*Lower Right*) from NMO are presented along with morphologically identified plasma cells ($CD19^{int}CD27^{high}CD38^{high}CD138^{+}$) from HS (*Upper Right*).

morphologically indistinguishable from the $CD138^{-}$ population in NMO patients or HS, indicating the immature characteristic of $CD27^{high}CD38^{high}CD180^{-}$ cells (Fig. S6). These phenotypical and morphological characteristics as well as the results of the quantitative real-time PCR (qRT-PCR) analysis indicate that this B-cell population is equivalent to plasmablasts (PB) (22–26). Hereafter, we use the term "PB" to distinguish this population from other B cells.

Expression of B-Cell Cytokine Receptors on PB. Prior studies have identified cytokines that are critically involved in the differentiation and/or survival of plasma cells, including IL-6 and B-cell–activating factor (BAFF). IL-6 induces B-cell differentiation into plasma cells, maintains early plasma cell survival, and enhances plasma cell IgG secretion (24). Besides, IL-6 is elevated in the cerebrospinal fluid (CSF) or peripheral blood of NMO patients compared with that of CMS patients and HS (27, 28). In a rodent autoimmunity model, IL-6 deficiency caused impaired autoantibody secretion by B cells (29). Given the potential role of IL-6 in NMO, we performed flow cytometry analysis for the expression of IL-6R. Results showed remarkable expression of IL-6R on PB, although it was only marginal or absent on mB and nB (Fig. S7). Because BAFF and A proliferation-inducing ligand (APRIL) can also promote the survival of PB (25, 26), we next evaluated the expression of the receptors for BAFF and APRIL, BAFF receptor (BAFF-R), B-cell maturation antigen (BCMA), and transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI). Expression of BCMA and TACI was selectively up-regulated in PB in parallel with IL-6R. In contrast, BAFF-R was up-regulated in mB and nB, but not in PB (Fig. S7).

PB Is a Selective Source of AQP4-Abs in Peripheral Blood. We were interested to know whether PB were capable of producing AQP4-Abs upon stimulation with cytokines and, therefore, examined the ability of IL-6, BAFF, and APRIL to enhance AQP4-Ab secretion by PB. We cultured the isolated PB for 6 d in the presence or absence of each cytokine, and evaluated the presence of AQP4-Abs in the supernatants by measuring IgG binding to Chinese hamster ovary (CHO) cells transfected with the human AQP4 vector (CHO^{AQP4}) or the vector control (CHO^{VC}). We found that IL-6, but not BAFF or APRIL, could significantly enhance AQP4-Ab secretion from PB (Fig. S8), as assessed by specific IgG binding to CHO^{AQP4}. Further study focusing on IL-6 showed that exogenous IL-6 promoted the production of AQP4-specific IgGs from PB (Fig. 3*A*), but not from the other B-cell subpopulations. Similar results were obtained from six independent experiments (Fig. S9), indicating that PB could be major AQP4-Ab producers in PBMC. In the absence of addition of IL-6, supernatants from PB did not show any significant reactivity to CHO^{AQP4}. To further analyze the AQP4-Ab–secreting potential of each B-cell subpopulation, we next stimulated the cells with a combination of IL-6, IL-21, and anti-CD40 that efficiently induces B-cell differentiation and IgG production (30). This polyclonal stimulation induced the secretion of similar amounts of IgGs from mB and PB. However, only the supernatant of PB specifically reacted to CHO^{AQP4} cell transfectants, indicating that AQP4-Ab–producing B cells were highly enriched in PB (Fig. 3*B*).

Survival and Functions of PB Depend on IL-6 Signaling. We evaluated the influence of IL-6, BAFF, and APRIL on the survival of PB after 2 d of in vitro culture (Fig. 4*A*). Among the added cytokines, only IL-6 was found to significantly promote the survival of PB (Fig. 4*B*). We also assessed the expression levels of X-box-binding protein 1 (XBP-1) in PB by qRT-PCR after 24 h of culture with or without IL-6. XBP-1 is a transcription factor critical for IgG secretion (31), and the splicing process of XBP-1 mRNA yields a more active and stable protein. We found that the expression of both unspliced [XBP-1(u)] and spliced [XBP-1(s)] forms of XBP-1 mRNA was augmented in PB by the ad-

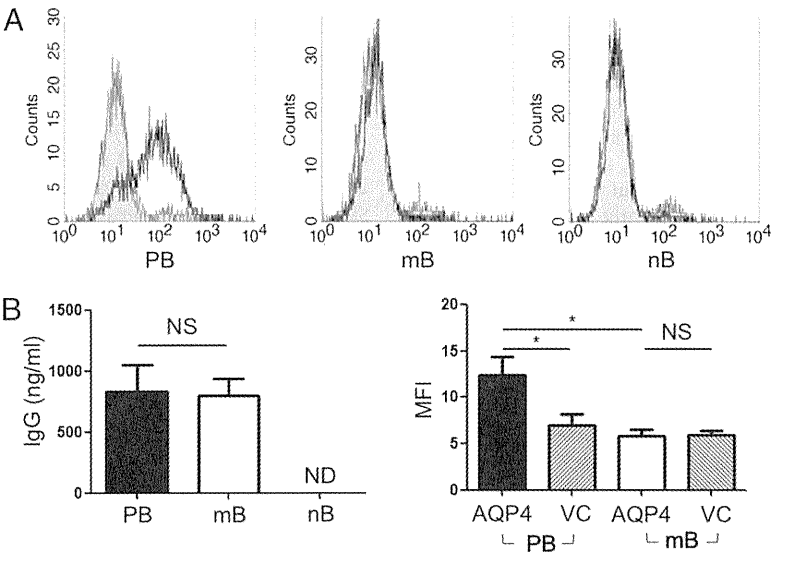

Fig. 3. Production of AQP4-Abs by PB. (*A*) Using flow cytometry, we examined whether AQP4-Abs could be produced by PB, mB, or nB cells. FACS-sorted cells were cultured with IL-6 (1 ng/mL) for 6 d and supernatants were collected. Supernatant IgGs reactive to CHO^{AQP4} (open histogram) and CHO^{VC} cells (closed histogram) were detected by anti-human IgG secondary antibody. The supernatant from PB (*Left*), but not from mB or nB, contains IgGs reactive to CHO^{AQP4}, indicating that only PB secrete AQP4-Abs after stimulation with IL-6. (*B*) Memory B cells (mB) produce IgGs but not AQP4-Abs. B-cell subpopulations were cultured in the presence of IL-6 (1 ng/mL), IL-21 (50 ng/mL), and anti-CD40 mAb (1 μg/mL) for 6 d. IgGs in the culture supernatants were measured by sandwich ELISA (*Left*) (each assay was performed in quadruplicate). Data from three patients are expressed as mean ± SD. The activity of AQP4-Abs in the culture supernatants from PB and mB was also measured by flow cytometry (*Right*). Aliquots of CHO^{AQP4} cells (AQP4) and CHO^{VC} cells (VC) (n = 4 for each) were stained with the supernatant of PB or mB from every patient. Data are expressed as median fluorescence intensity values from the results of three patients (*$P < 0.05$; Tukey's post hoc test). ND, not detected; NS, not significant.

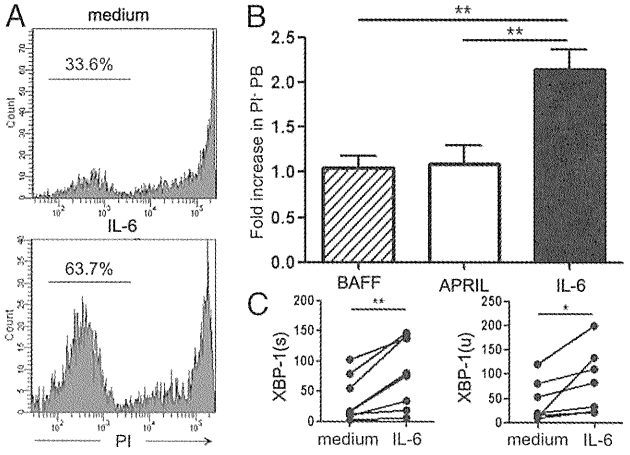

Fig. 4. Effect of exogenous IL-6 on PB. (*A*) IL-6 promotes the survival of PB. FACS-sorted PB were cultured in the presence or absence of recombinant IL-6 (1 ng/mL) for 2 d. PI staining of cultured PB showed that exogenous IL-6 increased the percentage of surviving cells (*Lower*) compared with cells cultured in the medium alone (*Upper*). Values shown are percentages of unstained cells. (*B*) Comparison of IL-6 with BAFF and APRIL. Here we show that only IL-6 could promote cell survival. Data are expressed as fold increase of % PI^- cells following the addition of each cytokine. At least four independent experiments were performed to obtain the results (**$P < 0.01$; Tukey's post hoc test). (*C*) Effect of IL-6 on XBP-1 expression. FACS-sorted PB were cultured with or without IL-6 for 24 h, and total RNA was extracted from the cells to quantify expression levels of XBP-1(u) and XBP-1(s) by qRT-PCR. Each line connects values obtained from seven independent experiments (*$P < 0.05$; **$P < 0.01$; Wilcoxon signed-rank test).

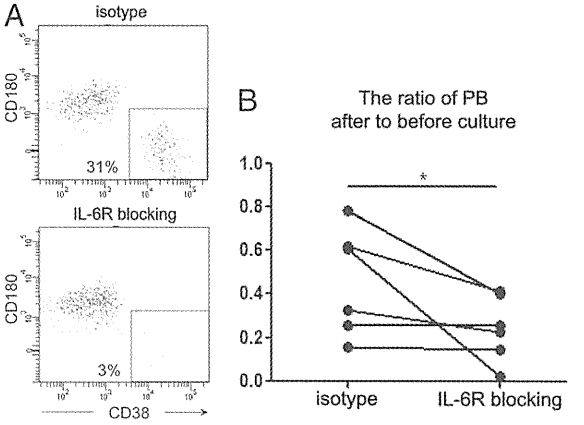

Fig. 5. IL-6R blockade selectively inhibits the survival of PB. (*A*) PBMC were cultured in a medium containing 20% autologous serum in the presence of IL-6R–blocking antibody or its isotype control mAb for 2 d. The cells were stained and analyzed as described in the experiment in Fig. 1*A*. Data represent the percentages of PB within $CD19^+CD27^+$ cells. A representative pair of six independent experiments is shown. (*B*) The percentage of PB within $CD19^+$ B cells (PB%) was determined for each pair of cultures either with anti–IL-6R mAb (IL-6R–blocking) or isotype control mAb (isotype) before and after starting the culture. Then, the PB survival ratio was calculated for each culture by dividing the PB% at the end of the culture by the PB% obtained before starting the culture. Lines connect the PB survival ratios of six independent experimental pairs to clarify that IL-6R blockade reduces PB survival (*$P < 0.05$; Wilcoxon signed-rank test).

dition of IL-6. These results suggest that IL-6 promoted the survival of PB and enhanced IgG secretion from PB, leading to an increased production of AQP4-Abs in NMO patients (Fig. 4*C*). In addition, we found that the frequency of PB tended to be increased when serum IL-6 levels were higher than the mean ± 2× SEM of those in HS [PB/PBMC (%) for IL-6 high group 0.62 ± 0.47 (%); PB/PBMC (%) for IL-6 low group 0.15 ± 0.05 (mean ± SD)]. These observations prompted us to address whether the blockade of IL-6R signaling could exhibit any influence on PB. We cultured PBMC derived from AQP4-Ab seropositive patients in the presence of 20% autologous serum and examined the effect of adding anti–IL-6R antibody by counting the number of surviving PB. We found that the frequency of PB among total B cells decreased significantly in the presence of anti–IL-6R mAb (Fig. 5 *A* and *B*). Among six patients examined, the PB reduction was remarkable in three patients, but was only marginal in the other patients. Notably, the former group of patients showed higher IL-6 levels in the serum (4.69, 6.47, and 25.5 pg/mL for each patient), compared with the latter (1.42, 1.43, and 2.91 pg/mL). The frequency of other B-cell subpopulations did not change with the addition of anti–IL-6R mAb. These results led us to postulate that in vivo administration of anti–IL-6R mAb may ameliorate NMO.

Discussion

A growing body of evidence suggests that AQP4-Abs play a pathogenic role in NMO (6, 7, 10–12). Here we report that a B-cell subpopulation bearing the $CD19^{int}CD27^{high}CD38^{high}CD180^-$ phenotype is responsible for the selective production of AQP4-Abs. The cells that we call PB are vulnerable to IL-6R blockade by anti–IL-6R mAb, leading us to propose anti–IL-6R mAb as a therapeutic option for NMO. Bennett et al. recently reported that plasma cells in CSF are a potential source of pathogenic AQP4-Abs (10). However, this study has not excluded a possible role of AQP4-Abs produced in the peripheral blood. It has been repeatedly shown that the passive transfer of pathogenic autoantibodies, including AQP4-Abs (10–12, 32), augments the formation of inflammatory lesions in EAE. Therefore, once T-cell–mediated inflammation takes place in the CNS, pathogenic autoantibodies produced outside the CNS are able to enter the CNS compartment. It is also notable that AQP4-Abs are more abundant in the peripheral blood of NMO patients than in their CSF (33). Taken together, we speculate that PB that are expanded in the peripheral blood during relapse may play a critical role in the pathogenesis of NMO by producing AQP4-Abs, although more work is necessary to explore whether PB can enter the CNS.

It is generally thought that circulating IgGs are mainly secreted by long-lived plasma cells residing in healthy bone marrow. It remains unclear how PB secreting AQP4-Abs can differentiate and survive in the peripheral circulation. It has been previously shown that autoantibodies producing plasma cells accumulate in peripheral lymphoid organs (34). It would be interesting to investigate which organs blood PB move to during the course of NMO. The levels of IL-6 in the serum and CSF are elevated in NMO compared with HS or CMS patients (27, 28). In this regard, it is of note that blocking IL-6R signaling was found to dramatically reduce the survival of PB ex vivo, which was dependent on the presence of autologous serum containing IL-6. These results suggest that the increase of PB in AQP4-Ab seropositive patients could be attributed to the increased IL-6 in the serum. We also demonstrated that improved PB survival in the presence of exogenous IL-6 was accompanied by up-regulated expression of XBP-1. It is noteworthy that wild-type and $XBP\text{-}1^{-/-}$ B cells start to produce more IL-6 after forced overexpression of XBP-1(s), which results in the operation of a positive feedback loop controlling IgG secretion (31). Treatment with anti–IL-6R is promising because IL-6R blockade could terminate this vicious loop that controls the production of autoantibodies.

It has been reported that NMO patients have higher levels of BAFF in the serum or CSF compared with CMS patients (35). BAFF is also known to support plasma cell differentiation and survival of PB induced in vitro (25). However, in our ex vivo

study, BAFF did not promote the survival of PB, indicating that PB were not a target for BAFF. We speculate that BAFF might specifically act on an early process of plasma cell differentiation and does not have an influence on cells like PB that have entered a later stage.

IL-6R blockade by humanized mAb against IL-6R (tocilizumab) has proven to be useful for treating immune-mediated diseases, including rheumatoid arthritis (36) and Castleman's disease (37). Here we propose that IL-6R–blocking antibody treatment should be considered as a therapeutic option for NMO. Currently, most NMO patients are being treated with corticosteroids in combination with immunosuppressive drugs and plasma exchange (38). Anti-CD20 mAb, which causes B-cell depletion, has also been used for serious cases of NMO. Because the level of B-cell depletion appears to correlate with the suppressive effects of anti-CD20 in NMO (39), it has been argued that B cells are essential for the pathogenesis of NMO, either via acting as antigen-presenting cells or as autoantibody producers. Weber et al. recently reported that activated antigen-specific B cells serve as antigen-presenting cells and polarize proinflammatory T cells in EAE (40), supporting the view that the therapeutic effects of anti-CD20 might be attributable to the depletion of antigen-presenting B cells. Notably, they also cautioned that elimination of $CD20^+$ cells might deplete nonactivated cells as well as regulatory B cells possessing anti-inflammatory potentials. Although the effect of anti-CD20 on AQP4-Ab–secreting cells has not been reported, it is likely that the majority of PB are not affected because they do not express CD20. Consistent with our prediction, anti-CD20 treatment was not effective in aggressive cases of NMO (41, 42). It appears that selective depletion of activated antigen-specific B cells could be a more promising strategy to improve the efficacy of B-cell–targeted therapies for NMO. In this regard, PB-targeting therapy is a promising approach. Given the efficacy of IL-6R blockade in reducing the number of PB ex vivo, we find it very interesting to explore the effect of anti–IL-6R mAb on NMO.

Materials and Methods

Patients and Controls. A cohort of 24 AQP4-Ab seropositive patients was recruited at the Multiple Sclerosis Clinic of the National Center of Neurology and Psychiatry (NCNP). Among these, 16 met the revised NMO diagnostic criteria (3). The other 8 were diagnosed with NMO spectrum disorder (1) because they did not develop both myelitis and optic neuritis (optic neuritis alone in 6 cases; myelitis alone in 2 cases). Seventeen age- and sex-matched CMS patients and 20 HS were enrolled as controls. Serum AQP4-Ab levels were measured by a previously reported protocol by courtesy of Kazuo Fujihara at Tohoku University (Sendai, Japan) (33). All CMS patients had relapsing-remitting MS and fulfilled McDonald diagnostic criteria (43).

At the time of blood sampling, 21 seropositive patients were receiving corticosteroids (prednisolone 5–25 mg/d). Seven of these patients were also being treated with azathioprine (12.5–100 mg/d) or tacrolimus (3 mg/d). Six CMS patients were receiving low-dose corticosteroids without immunosuppressants. None of the seropositive or CMS patients had received IFN-β, i.v. corticosteroids, plasma exchange, or i.v. immunoglobulins for at least 1 mo before blood sampling. Blood sampling during relapse was performed in six seropositive NMO patients before they received intensive therapy starting with i.v. corticosteroids. Five of these patients were followed up further and blood was collected again after they entered remission. Anti-nuclear and/or anti–SS-A Abs were detected in some of the seropositive patients, but none met the diagnostic criteria for SLE or Sjögren syndrome. Demographic features of the patients are presented in Table 1. The study was approved by the Ethics Committee of the NCNP.

Reagents. The following Abs were used in this study: mAbs against CD38, CD19, CD27, CD20, and PE-streptavidin (Beckman Coulter); mAbs against CD180 and BAFF-R (BD Biosciences); mAbs against IL-6R and TACI as well as Abs against BCMA and CD40 (R&D Systems); rabbit anti-human AQP4 antibody (Santa Cruz Biotechnology); FITC-anti-rabbit IgG (Jackson ImmunoResearch Laboratories); and FITC-anti-human IgG antibody (MP Biomedicals). Recombinant proteins of BAFF (ProSpec), APRIL (Abnova), IL-6 (PeproTech), and IL-21 (Invitrogen) were purchased. Propidium iodide (PI) was obtained from Sigma-Aldrich. RPMI 1640 supplemented with 10% FBS, 2 mM L-glutamine, 100 U/mL penicillin, and 100 mg/mL streptomycin (Life Technologies) was used for cell culture.

Flow Cytometry, Cytology, and Cell Culture. PBMC were separated using density centrifugation on Ficoll-Paque PLUS (GE Healthcare Biosciences). B cells were analyzed and sorted by FACSAria (BD Biosciences). Each B-cell subset was stained with May–Grünwald–Giemsa. To evaluate AQP4-Ab production in vitro, each B-cell subset (1 or 2×10^4) was cultured for 6 d in the medium alone, in the presence of IL-6 (1 ng/mL) or in the presence of IL-6 (1 ng/mL), IL-21 (50 ng/mL), and anti-CD40 (1 μg/mL). Culture supernatants were harvested and analyzed for AQP4-Ab production as described below. To examine the effect of cytokines on the survival of PB, the cells (4×10^3) were cultured in the medium alone or in the presence of BAFF (100 ng/mL), APRIL (300 ng/mL), or IL-6 (1 ng/mL) in 96-well U-bottom plates for 2 d and stained with PI to assess cell survival. In parallel, the cells were cultured for 1 d and harvested to evaluate mRNA expression by qRT-PCR. To assess the effect of IL-6 signaling blockade, PBMC (5×10^5) were preincubated with anti–IL-6R Abs (1 μg/mL) at 4 °C for 20 min, cultured in AIM-V medium (Invitrogen) containing 20% of heat-inactivated serum obtained from each patient in 96-well flat-bottom plates for 2 d, and analyzed by flow cytometry.

Quantitative RT-PCR Analysis. mRNA from each cell subset was isolated according to the manufacturer's instructions using an RNAeasy Kit (Qiagen). RNA was further treated with DNase using the RNase-Free DNase Set (Qiagen) and reverse-transcribed to cDNA using a cDNA synthesis kit (Takara Bio). PCR was performed using iQ SYBR Green Supermix (Takara Bio) on a LightCycler (Roche). RNA levels were normalized to endogenous β-actin (ACTB) for each sample. Primers used are listed in Table S1.

Measurement of Ig Isotypes and Serum IL-6. Secreted IgG in the culture supernatant was quantitated by sandwich ELISA using affinity-purified goat anti-human IgG-Fc (Bethyl Laboratories). Bound IgG was measured according to the manufacturer's instructions. Serum IL-6 was measured by ELISA (R&D Systems) according to the manufacturer's instructions.

AQP4-Ab Detection Assay. Human AQP4-expressing cells were established to detect AQP4-Abs by flow cytometry. A human AQP4 (hAQP4) M23 splice variant from a clone collection (Invitrogen) was amplified by PCR and subcloned into a pIRES-DsRed-Express vector (Clontech). CHO cells (American Type Culture Collection) were transfected with this hAQP4 M23 vector (CHO^{AQP4}) or vector

Table 1. Demographic features

	HS	Seropositive patients	CMS patients
Number	20	24	17
Age	44.7 ± 2.8	47.9 ± 3.2	41.3 ± 3.0
Male:female	5:15	1:23	5:12
Disease duration		12.0 ± 1.6	9.4 ± 2.4
Age of symptom onset		36.1 ± 3.0	31.9 ± 3.4
Relapses in last 2 y		1.4 ± 0.3	0.7 ± 0.2
EDSS score in disease remission		5.0 ± 0.5	2.1 ± 0.6
Other autoantibodies		ANA 13, SS-A 5	ND

Demographic features for HS, AQP4-Ab seropositive patients, and CMS patients. Values are expressed as number or mean ± SEM. ANA, anti-nuclear antibody; ND, not detected; SS-A, anti–SS-A antibody; EDSS, expanded disability status scale.

control (CHO^{VC}) using FuGENE 6 Transfection Reagent (Roche). After 2 wk of geneticin (Invitrogen) selection, stable clones were established by single-cell sorting. The expression of hAQP4 in the established clones was confirmed using anti-human AQP4 antibody and FITC-anti-rabbit IgG antibody. Reactivity of AQP4-Abs to CHO^{AQP4} was confirmed using seropositive NMO patients' sera diluted at 1:1,000 and FITC-anti-human IgG antibody. To measure the AQP4-Ab activity in culture supernatants, these supernatants were concentrated up to 10 times using an Amicon Ultra 0.5 mL 100K device (Millipore), and 10 μL of the solution was added to 3×10^4 CHO^{AQP4} and CHO^{VC} cells. After incubation on ice for 20 min, cells were washed with sterile PBS containing 1% BSA and stained with FITC-anti-human IgG antibody. After a 10-min incubation on ice, the cells were washed and fixed for 15 min in 2% paraformaldehyde. Then the cells were washed and analyzed by flow cytometry.

Data Analysis. Histogram overlay analysis was performed using Cell Quest software (BD Biosciences). Statistics were calculated using Prism (GraphPad Software). Wilcoxon signed-rank test, Mann–Whitney *U* test, ANOVA, or Spearman's correlation test were also used when appropriate. Post hoc tests were used as a multiple comparison test after confirmation of equal variances by ANOVA.

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スでは高血糖にも注意する．

5．血液浄化療法

二重膜濾過法や免疫吸着法を行うことが多い．血液浄化療法は，血液内より抗AChR抗体を除または吸着除去することにより比較的短期間で果を示す．クリーゼ時や手術前後の筋無力症状軽減を目的に行われる．効果は長期間持続するのではないため，ステロイドを含む免疫抑制薬併用することが望ましい．

6．免疫グロブリン大量療法

免疫グロブリン大量療法(intravenous immunoglobulin；IVIG)は，血液浄化療法のよう特別な装置を必要とせず，容易に施行できる．0 mg/kg/日の免疫グロブリンを5日間連続投する．現時点ではMGに対しての有効性は確しているが保険適用はない．

おわりに

MGにおける抗AChR抗体の病態への関与については比較的理解され，動物へのトランスファーも成功している．しかし，MGの発症メカズムはいまだに不明であり，胸腺の役割についも解明されたわけではない．抗AChR抗体陰のMGでは新規の自己抗体も報告されている．Gの病態を理解するためには，発症のメカニズ，自己抗体の種類と病態への関与，さらに抗体作用した後に起こる骨格筋の変化についても研を進める必要がある．

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Summary

Myasthenia gravis

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Myasthenia gravis (MG) is an autoimmune disease associated with the anti-acetylcholine receptor (AChR) antibodies and nearly 80% of patients have these autoantibodies. In MG, thymus and thymoma play important roles in central tolerance and result in autoimmunity. Muscle specific receptor tyrosine kinase (MuSK) and low-density lipoprotein receptor-related 4 protein (Lrp4) are necessary components of the cluster of AChR on the neuromuscular junction. In MG, there are the antibodies against MuSK and Lrp4, and these antibodies and AChR antibodies are definitely pathogenic. To develop new therapies for MG, we have to carry out the continued research for the mechanism and pathogenesis of the disease.

〔Rinsho Kensa 55 : 1234-1240, 2011〕

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4．そのほかの自己免疫疾患
6）多発性硬化症・視神経脊髄炎

千原典夫[1]/山

SUMMARY

中枢神経の炎症性自己免疫疾患の代表である多発性硬化症(MS)と視神経脊髄炎(NMO)では，T細胞やB細胞の介在する炎症病態が詳細に解析され，新しい治療法の開発につながる研究成果が挙がっている．中枢神経組織は通常血液脳関門(BBB)によって保護されているが，一度，神経細胞が障害されると自己再生能に乏しいために，徐々に神経脱落症状が蓄積する．MSやNMOの自己免疫病態を考えるうえで，標的臓器である中枢神経の特殊性を考慮しなければならない．一方で，血液リンパ球を用いた解析は次々に新しい知見を生んでおり，他の自己免疫疾患の研究にも影響を与えている．本稿では，MSおよびNMOにおけるその病態の特徴や両者の違いについて最新の知見を紹介する．

〔臨床検査　55：1241-1248，2011〕

KEYWORDS

血液脳関門，フローサイトメータ，病原性T細胞，プラズマブラスト

はじめに

多発性硬化症(multiple sclerosis；MS)と視神経脊髄炎(neuromyelitis optica；NMO)は中枢神経を標的とする自己免疫疾患のなかで，詳細な病態解析が行われ，この分野の研究や新規治療薬開発をリードしている．

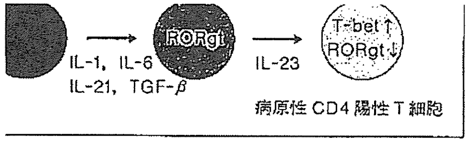
本稿では，MSとNMOの病態を概説し，両者の違いと治療法について述べる

本邦における研究(

1930年代に東京帝国大学の三浦ツの学会で，「日本にはMS患者にしない」と報告した．しかし，19ら[1]は，欧米より少ないながら日本は存在し，大脳病変を主体とする脊髄病変を主体とする患者が約半数両者間の移行もみられるとした．病変を主体とする患者は欧米では極めから，“アジア型MS”という呼びるようになった．今日では，“アジ大部分は，抗アクアポリン4(AQP4)抗体が関与するNMOであかになっている．

日本での患者数は特にMSで近り，現在MSとNMOをあわせたての認定者数は約13,000人に上りMSとNMOで7：3程度である．率は内外で大差がないのに対し，Nではその有病率が日本の10倍あが近年増加している理由として，タイルの欧米化などの後天因子がの他MRIなどの診断技術が向上しの1つであろう．

MSとNMOは病勢の再発と寛ため，かつてこれらは同一疾患の亜られ，同様の治療が施されてき



病原性 T 細胞の分化
' 細胞は抗原提示細胞によって様々なサイトカイン刺受け，ナイーブ T 細胞から転写因子 RORgt を発現化する．その後 IL-23 の刺激を受け活性型となるが，過程で転写因子 T-bet が上昇し IFNγ 産生性を獲得と中枢神経への浸潤能を有する病原性 T 細胞になるれている．
-β：transforming growth factor-β.

と NMO は区別されず，MS として難病指定けている．しかし，2000 年頃より日本でもしてきたインターフェロン β(IFNβ) 治療視神経脊髄型 MS（すなわち現在の NMO）で分な効果が得られないなど，診療のうえでひとめにできない状況が明らかになってきた．

年に Mayo Clinic の Lennon ら[2] によって，経脊髄型 MS 患者の血清 IgG はアストロサに特異的に反応することが報告され，さらに抗体がアストロサイト上に高発現する水チャである AQP4 に対する自己抗体であることらかとなり，両者の鑑別が容易になったこと抗 AQP4 抗体陽性の症例を NMO としてから分離する考え方が急速に広がった．その両者の病態の違いについての解析が進んでい

MS の病態

S は脳・脊髄・視神経の複数領域に炎症性病多発し，再燃を繰り返しながら年余にわたっ変数が増加し，それに伴い大脳萎縮などが現疾患で，若年の女性に発症する傾向が強い．診断には "時間的" 多発性（再発すること）お "空間的" 多発性（中枢神経の複数か所に病疑われること）に加えて，他疾患が除外されとが必要である[3]．今日では MS は中枢神経鞘に由来するミエリン抗原（ミエリン塩基性

胞やマクロファージの浸潤に加え，オリゴデンドロサイトや神経髄鞘の脱落が著明であり，アストロサイトや神経軸索は比較的保たれている．自己免疫病態の中心を担うのは CD4 陽性ヘルパー T 細胞（Th 細胞）であるが，CD8 陽性 T 細胞の関与も報告されている．ミエリン抗原特異的に反応する Th 細胞の指令を受け，マクロファージなどの炎症細胞が活性化され髄鞘障害を起こす．重症例では軸索も障害されることがわかってきたが，あくまで髄鞘障害が前景に出るので，脱髄疾患として問題ない．

MS の動物モデルである自己免疫性脳脊髄炎 (experimental autoimmune encephalomyelitis；EAE) では，長年にわたってインターフェロン γ(IFNγ) を産生する Th1 細胞が中心的な役割を果たすとされてきたが，近年では Th1 細胞に加えて，インターロイキン 17(IL-17) 産生性の Th17 細胞が病原性 T 細胞として重要であるとされる．Th17 は抗原提示細胞から供給されるインターロイキン 23(IL-23) の刺激を受けて活性型に変化し，*IL-23* 欠損マウスでは EAE は発症しないことが示されている．

最近，Hirota ら[4] は IL-17A のレポーターマウスを用いて Th17 細胞の EAE 発症における動態を詳細に解析した．ミエリン抗原の 1 つであるミエリンオリゴデンドロサイト糖蛋白 (myelin oligodendrocyte glycoprotein；MOG) に特異的な Th17 細胞は，炎症が進むにつれて IFNγ の産生能が増し，IFNγ 産生細胞や一部は IFNγ と IL-17 とのダブルプロデューサーとなり病原性を発揮することを示した（図 1）．

IL-23 欠損マウスでは IFNγ 産生能を獲得する過程が障害される．IL-17 産生 T 細胞として誘導された T 細胞が，IFNγ 産生能を獲得することによってはじめて，脳炎惹起性を発揮する可能性が示された．この IFNγ 産生性の獲得には IFNγ 産生細胞に発現する転写因子 T-bet が Runx-1 と結合して，Th17 の master regulator（その T 細胞分化に最も重要である転写因子）で

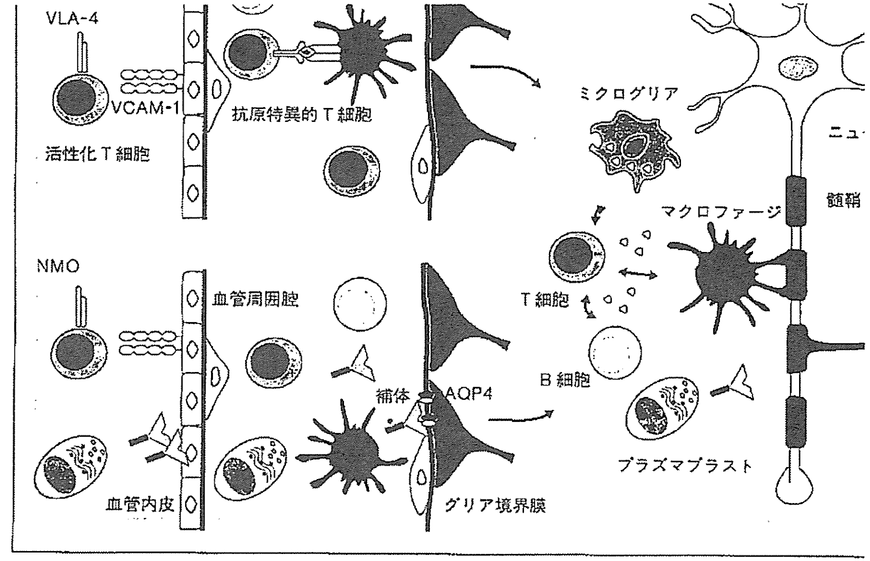


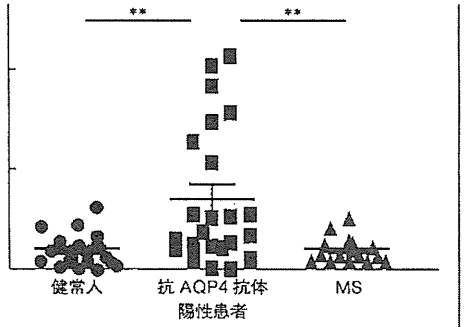
図 2　MS と NMO における BBB 破綻メカニズムの違い
BBB(blood-brain barrier) は血管内皮とグリア境界膜の 2 層構造からなる．活性化 T 細胞は VLA 因子を用いて血管内皮を通過するが，グリア境界膜を越えるには，血管周囲腔で抗原提示細胞に再必要がある．ミエリン抗原特異的な T 細胞は中枢神経組織へ浸潤しマクロファージ，ミクログリアと共同し髄鞘障害を起こす．NMO(neuromyelitis optica) では抗 AQP4(aquaporin 4) 抗体がグリ AQP4 に結合しアストロサイト障害を引き起こすと考えられるので，活性化 T 細胞などによる炎症内皮の透過性が増せば，抗 AQP4 抗体を発動因子として中枢神経組織障害が惹起される．

ている[5]．一方で，この病原性 T 細胞がどこで活性化されて，どのように髄鞘障害を起こすのかはいまだ明らかでない．

MS においてリンパ組織で活性化された T 細胞をはじめとする炎症細胞が中枢神経内に達し，髄鞘障害を及ぼすためには血液脳関門 (blood-brain barrier；BBB) を越える必要があり，その過程が MS 病態の理解を複雑にしている．BBB は血管内皮とアストロサイトの足に裏打ちされた基底膜（グリア境界膜：glia limitans）の 2 層構造からなる（図 2）．非特異的な炎症で誘導された活性化 T 細胞は VLA-4(very late antigen-4) などの接着因子によって血管内皮上に発現した VCAM-1(vascular cell adhesion molecule-1) などと結合して血管内皮を透過するが，グリア境界膜は越えない．

近年 Bartholomäus ら[6] は，2 光子励起顕微鏡

ア境界膜を越えて脳実質へ浸潤す．アルブミン (ovalbumin；OVA) 特血管内皮を透過するもののグリアないことを示した．これは，血管も膜下腔に MBP を提示する抗原し，MBP によって再活性化されT 細胞はサイトカインやケモカイのに対して，OVA 特異的 T 細胞め再活性化されないからである．人為的にくも膜下腔に添加すると細胞もグリア境界膜を越えた．通取り込んだ抗原提示細胞はリンパ T 細胞に抗原を提示し，抗原特異性化を誘導する．くも膜下腔におれて脳実質に浸潤するということ胞が二段階の活性化を必要とするる．前述の IFNγ 産生能の獲得か



(**: $p<0.01$, Tukey's Multiple Comparison Test)

末梢血 B 細胞中のプラズマブラストの割合
血における B 細胞中のプラズマブラストの割合は，
QP4(aquaporin 4)抗体陽性患者で同年齢・性別の健
や MS 患者と比較して有意に増加していた(健常人：
，抗 AQP4 抗体陽性患者：24 例，MS 患者：17 例)．
〔文献 6)より転載〕

NMO の病態

MO はデビック病(Devic's disease)と呼ばれ
患群を包含するが，その異同については議論
い．ここでは抗 AQP4 抗体陽性で再発する
を典型的な NMO と定義して議論を進める．
MO は中枢神経系の中でも視神経と脊髄に重
炎症性病変を認める．極めて興味深いことに
の 9 割が女性で，発症年齢は 30 歳代と，
よりも若干高齢である．“時間的”および
的”多発病変の存在は MS の診断基準に合
，NMO の典型例においては，臨床経過，画
病理像が MS と明らかに異なる．まず臨床
は再発寛解型 MS と比較して重篤で，一度
発で高度の神経障害による後遺症状を残すこ
しばしばみられる．次に，脊髄 MRI 画像所
おいて，浮腫を伴い，3 椎体以上に及ぶ脊髄
(long spinal cord lesion)がみられる．病理
は MS でみられる髄鞘の選択的脱落ではな
血管周囲に広がり壊死性病変を伴う非選択的
障害が特徴である．しかし，視神経や脊髄に
はグリア境界膜に高発現する AQP4 を認識する．
NMO の病理では血管周囲に IgG と補体の沈着
がみられ，沈着部位のアストロサイトは破壊され
る．初期病変にはアストロサイト上の AQP4 の
みが脱落していることもあり，抗 AQP4 抗体が
グリア境界膜を破綻させる先兵となりうることを
示している(図 2)．EAE を発症させたラットに
患者 IgG を静脈投与すると NMO 類似の病理像
を呈したことから，抗 AQP4 抗体がその疾患
マーカーとしてだけでなく，病態にも関与してい
ることが示唆されている．

抗 AQP4 抗体がグリア境界膜の破壊を誘導す
るということは，非特異的炎症であっても抗体が
血管内皮を透過する状態になれば，再発に至る可
能性があることを意味する．BBB を通過するた
めに血管周囲腔で抗原提示細胞に抗原特異的 T
細胞が再活性化される必要のある MS と比較し
て，NMO では AQP4 抗原以外の抗原に反応す
る非特異的な活性化 T 細胞であっても組織破壊
に関与する可能性があり，MS より広範な非選択
的組織障害を起こすのかもしれない．

抗 AQP4 抗体は主に IgG 抗体である．これは
リンパ組織において AQP4 特異的 T 細胞によっ
て活性化された AQP4 特異的 B 細胞の存在を意
味する．筆者ら[7]は最近，NMO の末梢血に未熟
形質細胞(プラズマブラスト)が増加していること
を発見した．プラズマブラストは末梢血における
主な抗 AQP4 抗体産生細胞であり，再発時に増
加していた．MS や健常人ではこの増加は認めら
れなかったため，プラズマブラストの増加は
NMO に特異的な B 細胞異常であると考えられ
る(図 3)．

なぜプラズマブラストが増加するのかは今後の
検討課題であるが，NMO では抗原特異的な活性
化 B 細胞が決定的にその病態に関与している可
能性が高い．かつて NMO において IFNβ によ
る治療が奏効しなかったのは，IFNβ には B 細
胞分化を促進する作用があり，病原性 B 細胞を
活性化させてしまっていたためだと考えられる．

これまで述べてきたように，MS と NMO は
その鍵となる病態が異なる．臨床の場においては
その鑑別が重要である．これは現在 MS の寛解
維持療法として用いられる IFNβ が効果を示す
か否かの鑑別とも言い換えられる．IFNβ は MS
の病態に重要な病原性 T 細胞(Th17 や IFNγ 産
生細胞)の分化抑制効果を有する一方で，NMO
では抗 AQP4 抗体などの自己抗体を産生する病
原性 B 細胞の分化を促進する．

現在 NMO の診断基準(Wingerchuk 2006：視
神経炎と横断性脊髄病変の共存)を満たさず，視
神経炎もしくは脊髄病変しか認められないが，抗
AQP4 抗体陽性の例について NMO spectrum
disorder として同様の病態と扱う考え方がある[9]．抗 AQP4 抗体の特異性は高く，その病態機
序から抗体陽性例は同様の病態としてよいと考え
られる一方で，NMO における抗 AQP4 抗体の
感度は 30～80％と報告によってばらつきがある．
標準的な抗 AQP4 抗体測定法は AQP4 蛋白を強
制発現させた培養細胞を用いて，患者 IgG の反
応性を間接蛍光抗体法で染色し測定する．そのほ
か，AQP4 蛋白を用いた sandwich ELISA
(enzyme-linked immunosorbent assay)の手法があ
り，市販されている．感度のばらつきには測定
系の違いの影響があるかもしれないが，同様の病
態であっても抗 AQP4 抗体が陰性で，例えば他
の病原性をもつ自己抗体を有する可能性も考えな
ければならない．実際，抗 AQP4 抗体陰性の
NMO 例や，臨床的には MS と診断されても
IFNβ の効果がない MS 例があり，治療に難渋
することがしばしばである．

MS の診断バイオマーカーとして，自己抗体以
外には髄液オリゴクローナルバンド(oligoclonal
bands；OCB)がある．OCB の発見は 60 年前に
遡るが，その病態における位置づけはいまだ明確
でない．OCB は等電点電気泳動法により測定さ
れ，MS において感度 86.5％，特異度 90％と診
断に有用である[10]．OCB の存在は髄腔内での B
なる[7]．しかし，OCB は抗 AQP4
あったように，NMO と MS を明
ものではない．正確な病態把握に
たバイオマーカーが必要である．

血清や髄液中の蛋白測定ではな
トメータ(fluorescence-activated
FACS)を用いて病原性細胞や制
解析する方法が複数報告されてい
モカイン受容体 CXCR3 や CCR5
細胞が MS 患者の髄液や炎症組織
り，病勢を反映するという報
CXCR3 や CCR5 は IFNγ 産生性
ケモカイン受容体である．その
は IFNγ 産生性細胞や IL-17 産生
しており，再発時には IL-17 産生
増加するという報告もある[12]．い
される T 細胞を直接解析してお
反映していると考えられる．また
を調節する細胞の異常も知られて
高発現する NK(natural killer)細
性細胞として知られる．この NK
末梢血で減少しており，病勢と逆
されている[13]．NMO においても
てプラズマブラストが抗 AQP4 抗
病勢を反映することがわかった(
抗 AQP4 抗体陰性 NMO は含め
後，解析を進めることで同様の
る 1 群が明らかになる可能性があ
FACS を用いて病態を反映する細
うことは，MS と NMO をその病
めに必要な次世代ツールである．

病態に応じた治療

MS および NMO の治療で問題
IFNβ の効果がない患者である．
はステロイドや免疫抑制剤を用い
われているが副作用も多く，より
療法が求められている．そこで

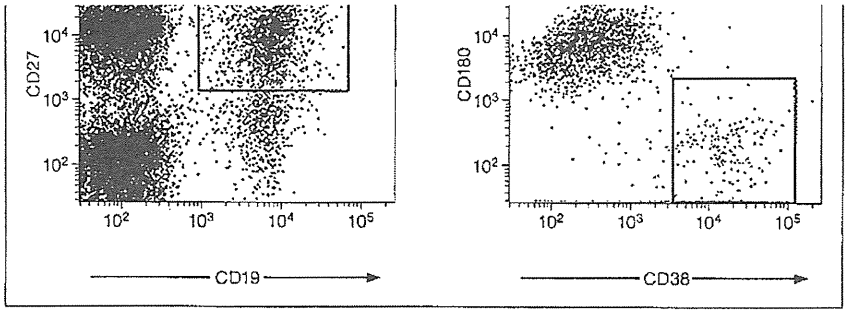


図4　フローサイトメータ(FACS)による解析
フローサイトメータ(FACS)による末梢血単核球細胞(peripheral blood mononuclear cell；PBMC)の解析例を示す．図の左はPBMCから表面マーカーのCD19，CD27陽性細胞を抽出したもので，四角で囲まれた部分はCD19陽性，CD27陽性細胞となる．さらに，この細胞を同時に染色したCD38，CD180の染色性で展開するとCD38陽性，CD180陰性のプラズマブラストの割合が算出できる．

〔文献6)より転載〕

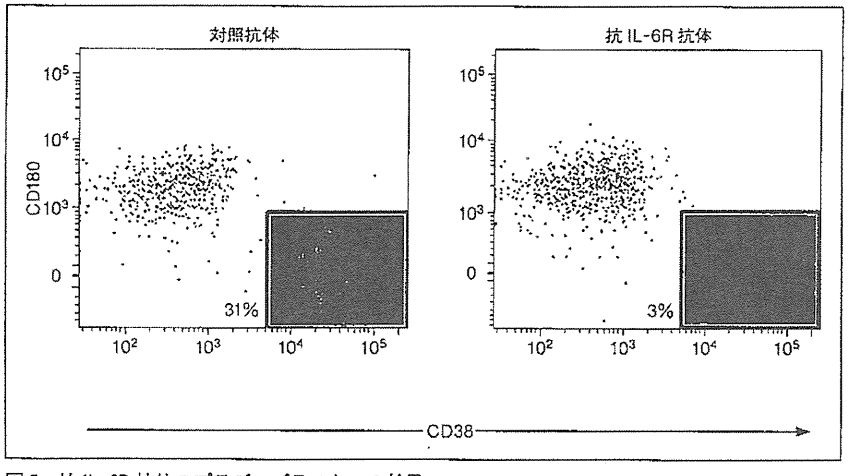


図5　抗IL-6R抗体のプラズマブラストへの効果
抗AQP4(aquaporin 4)抗体陽性患者末梢血単核球細胞(peripheral blood mononuclear cell；PBMC)を血清存在下に培養し，抗IL-6R抗体および対照抗体を加えた．図4で示したFACSの解析図と同様に展開した図を示す．抗IL-6R抗体を加えた場合にプラズマブラストが著明に減少していることがわかる(数値はCD19陽性，CD27陽性細胞中のプラズマブラストの割合を示す)．

〔文献6)より転載〕

T細胞の血管内皮への接着を阻害して中枢
経への浸潤を阻止する．しかし，非選択的に
細胞の接着を阻害するため，JCウイルスの中
枢神経内増殖を許し，進行性多巣性白質脳症を起
こすなどの合併症がある．スフィンゴシン1リン
経への浸潤を阻害する機序でMSに効果が期待
できるが，白血球減少などの合併症も予想され，
投薬管理が今後の課題である．FTY720は経口
薬であり，患者のQOL改善に期待がもたれる．

B細胞を標的とした抗CD20抗体によるB細
抗原提示細胞としてB細胞が重要であると考え
られている[14]．抗CD20抗体療法は本邦において
カリニ肺炎の合併が複数報告されており，思わぬ
副作用を生む可能性がある．

また，NMOへの効果が限定的であった理由と
して，抗AQP4抗体産生細胞がCD20を発現し
ていないことが挙げられる．NMOの病態に適し
た治療法は抗AQP4抗体産生細胞を標的とする
のがよいと思われる．抗AQP4抗体産生細胞の
生存や抗体産生能は主にインターロイキン6(IL-
6)シグナルによって制御されていることがわかっ
た．NMOの髄液や血清中にはIL-6が増加して
おり，病態にかかわっていると考えられる．IL-
6受容体(IL-6R)を阻害する抗IL-6R抗体は抗
AQP4抗体産生やプラズマブラストの生存を抑制
した(図5)．抗IL-6抗体療法は関節リウマチや
Castleman病に用いられ効果を挙げている．易
感染性などの副作用があるが，関節リウマチなど
の自己免疫疾患に対し本邦でも投与されているこ
とから，安全性は高いと思われ，治療法としての
普及が望まれる．

おわりに

MSとNMOについて，その基礎的な病態の
違いや臨床での問題点を中心に述べてきたが，
heterogenousな病態に対するテーラーメイド治
療を考えるうえで，FACSなどを用いてそれぞ
れの病態を可視化する研究がいっそう望まれる．
自己免疫疾患に関与する免疫細胞はT細胞，B
細胞，NK細胞など数多く，その中から病態の鍵
となるプレイヤーを見つけ出すことが病態に特化
した真の治療法に繋がると期待される．

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ie differential pathogenesis of multiple scle-
sis and neuromyelitis optica

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ıltiple Sclerosis (MS) and neuromyeltis Optica
MO) are autoimune neuroinflammatory diseases
ecting the central nervous system (CNS). In MS and
MO, the inflammatory pathogenesis mediated by T or
ymphocytes has been intensively investigated, which
earch has opened the gate to the designing of new

ever, because of the low regenerative potential of neuronal cells following severe inflammation, defects in neuronal structure and functions tend to accumulate over the years. Here we describe the differences in the pathogenesis of MS and NMO based on recent progress in this research field.

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4．そのほかの自己免疫疾患
7）Guillain-Barré 症候群

駒ヶ嶺朋子[1]/結

SUMMARY

C. jejuni（*Campylobacter jejuni*）腸炎後に発症する軸索型 Guillain-Barré 症候群は，分子相同性による抗体産生機序が立証された最初の自己免疫疾患である．患者から分離された *C. jejuni* のリポオリゴ糖はヒト神経組織に存在する GM1 ガングリオシドと糖鎖相同性を有していた．ヒト組織に類似した構造をもつ病原体に感染することにより病原抗体である抗ガングリオシド抗体が誘導され，神経組織の傷害が起こり弛緩性四肢麻痺に至る．抗ガングリオシド抗体の病的意義の確立は，抗体の種類による Guillain-Barré 症候群とその類縁疾患の分類再編を支持する．

〔臨床検査　55：1249-1257，2011〕

KEYWORDS

Guillain-Barré 症候群，分子相同性，ガングリオシド

はじめに

Guillain-Barré 症候群（Guillain-Barré syndrome；GBS）は，急性に発症する四肢筋力低下を主徴とする末梢神経疾患である．発症率は人口10万人当たり 0.4～4.0 人（中央値 1.3 人）であり，急性に発症する弛緩性麻痺のなかで最も頻度が高い[1]．多くの症例で発症の 1～2 週前と比較的短期間のうちに下痢など胃腸炎症状や上気道感染症状が先行することから，感染を引き金とした自己免疫機序による発症が以前から考えられてきた．

GBS は末梢神経の髄鞘が傷害さ
患と長年捉えられてきた．しかし，
的に軸索が傷害されるものも認識
り，脱髄型（acute inflammatory
polyradiculopathy；AIDP）と
motor axonal neuropathy；AMA
れる．AIDP は，電気生理学的検
認め，病理学的にはシュワン細胞
着，続く小胞性ミエリン変性を認
ヨーロッパや北米ではこちらが多

一方で，わが国や中国など
AMAN が半数を占め，末梢神経
グリオシドに対する抗体が陽性
AMAN において，患者から実際
原体を用いて疫学的および生化学
積され，疾患のモデル動物の樹立
染病原体とヒト組織との分子相同
体を介した神経症状の発症が立証

眼球運動障害，運動失調，腱反
する Fisher 症候群（Fisher syndr
た，抗ガングリオシド抗体が陽性
類縁疾患である．そのほか，四肢
障害や感覚障害をきたすものなど
亜型が存在する．患者血清の抗ガ
体の分析が，これらの臨床亜型の
現在では GBS 関連疾患の診断補
オシド抗体検索が汎用されている．
感染の病原微生物の解析により，