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Ganglioside-specific IgG and IgA recruit leukocyte effector functions in Guillain–Barré syndrome

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Abstract

The capacity of ganglioside-specific autoantibodies to recruit leukocyte effector functions was studied. Serum samples from 87 patients with Guillain–Barré (GBS) or Miller Fisher syndrome (MFS), containing GM1-, GQ1b-, or GD1b-specific IgG or IgA, were tested for leukocyte activating capacity. Ganglioside-specific IgG antibodies generally induced leukocyte activation, irrespective of specificity. The magnitude of leukocyte degranulation correlated with GM1- and GQ1b-specific IgG titers, but not with disease severity. Finally, GM1-specific IgA activated leukocytes through the IgA receptor, FcαRI (CD89). Therefore, both ganglioside-specific IgG and IgA can recruit leukocyte effector functions, which may be relevant in the pathogenesis of GBS and MFS.

Keywords: Guillain–Barré syndrome; Autoantibodies; Pathogenicity; Gangliosides; FcγR

1. Introduction

The cellular and humoral arms of the immune system are implicated in the pathogenesis of Guillain–Barré syndrome (GBS) (Hughes and Cornblath, 2005). Ganglioside-specific autoantibodies are detected in serum from approximately 40% of patients with GBS (reviewed in Willison and Yuki, 2002). These autoantibodies are of all major isotypes (IgM, IgG1, IgG3, and IgA) and display a wide range of specificities. Gangliosides are abundantly expressed and display a unique distribution pattern in peripheral nervous tissue, e.g. GD1b is enriched in dorsal root ganglion neurons and GQ1b in oculomotor nerves (Chiba et al., 1997; Gong et al., 2002). Correspondingly, antibody specificity is associated with specific clinical deficits, i.e. GQ1b-specific antibodies with ophthalmoplegia and Miller Fisher syndrome (MFS), GQ1b- and GD1b-specific antibodies with ataxic neuropathy, and GM1-specific antibodies with pure motor variants of GBS (Willison and Yuki, 2002).

Recent studies focused on the pathogenic properties of ganglioside-specific antibodies. Ganglioside-specific antibodies activate complement, which results in dysfunction and destruction of nerves or the neuromuscular junction

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(Goodyear et al., 1999; Halstead et al., 2004; Zhang et al., 2004). Activation of leukocytes may represent an additional immune mechanism contributing to demyelination or axonal damage. GM1-specific IgG in serum from GBS patients activates leukocytes via IgG receptors (FcγR) in the absence of complement (van Sorge et al., 2003b), and myelin-loaded macrophages are observed in nerve sections from autopsied patients with GBS (Hafer-Macko et al., 1996b). Furthermore, we observed that pro-inflammatory properties of GM1-specific antibodies, i.e. leukocyte and complement activation, in plasma from ganglioside-immunized rabbits are strongly associated with the development of axonal neuropathy (van Sorge et al., in press). Therefore, activation of leukocytes through antibody receptors (FcγR and IgA receptor (FcαRI, CD89)) by ganglioside-specific antibodies may contribute to the initiation or maintenance of inflammatory activity in nerves.

It is currently not known whether activation of leukocytes via FcγR is confined to IgG antibodies with specificity for GM1 (van Sorge et al., 2003a). Antibodies with other ganglioside specificities, including GQ1b and GD1b, are also frequently detected in serum from patients with GBS. In addition, GM1-specific IgA is detected in serum from patients with axonal degeneration and poor outcome (Ilyas et al., 1992; Koga et al., 1999). In this study, we investigated the leukocyte activating capacity of IgG antibodies with specificity for GM1, GQ1b and GD1b, and GM1-specific IgA.

2. Materials and methods

2.1. Patients and sera

Sera from 12 patients with GBS or MFS with high titers of GM1-, GQ1b- or GD1b-specific IgG or GM1-specific IgA were collected at the University Medical Center Utrecht, The Netherlands, and used for pilot experiments. In addition, 75 sera from patients with GBS or MFS (Table 1) were collected at the Dokkyo Medical University School of Medicine, Tochigi, Japan, containing GM1- ($n=26$), GQ1b- ($n=24$) or GD1b-specific ($n=25$) IgG with varying titers. One patient with GD1b-specific IgG antibodies had GBS with prominent ataxia, and three patients with GQ1b-specific IgG had isolated acute ophthalmoparesis (AO). These 75 sera were used to corroborate results from pilot studies and to study correlations between leukocyte activation, antibody titer, disease severity and clinical presentation (GQ1b only). Sera were obtained within two weeks after onset of weakness, before initiation of treatment, and stored at −80 °C until further use. The diagnosis of GBS was defined according to established criteria (Asbury and Cornblath, 1990), and the diagnoses of MFS and AO were defined according to previously published criteria (Odaka et al., 2001). Severity of GBS was scored as the patients' worst deficits at the peak of their illness using a previously described functional disability scale (van der Meché et al., 1992), referred to as Hughes' scale, on which 0 denotes healthy; 1, having minor symptoms and signs, and capable of walking; 2, able to walk ≥10 m without assistance; 3, able to walk ≥10 m with a walker or support; 4, bedridden or chair bound (unable to walk as described for grade 3); 5, requiring assisted ventilation for at least part of the day; and 6, dead. None of the patients, included in this study, died. Mild disease was defined as grades 1–3, severe disease as grades 4 and 5. All patients gave informed consent prior to testing. Serum from healthy blood donors, without ganglioside-specific antibodies as tested by enzyme-linked immunosorbent assay (ELISA), served as a negative control in all leukocyte degranulation experiments.

Table 1
Specificity of ganglioside-specific IgG and patient characteristics

Serum characteristics		Patient characteristics			
Serum samples (n)	Autoantibody specificity	Age (years, median (range))	Sex (M/F)	Diagnosis*	Disease severity (Hughes' scale, median (range))
26	GM1	36 (5–70)	13/13	26 GBS	3 (1–5)
24	GQ1b	52 (8–86)	17/7	17 MFS[1] 7 GBS	3 (0–5)
25	GD1b	41 (9–76)	12/13	24 GBS[2] 1 MFS	3 (1–5)

*MFS, Miller Fisher syndrome; GBS, Guillain–Barré syndrome. [1]Three patients with isolated acute ophthalmoparesis, [2]one patient with prominent ataxia.

2.2. Ganglioside-specific Ig ELISA

Ganglioside-specific antibody titers were assessed by ELISA as described, using mouse anti-human γ- or α-chain specific monoclonal antibodies (SBA, Birmingham, AL) (Yuki et al., 1997). Sera were considered positive for the presence of ganglioside-specific antibodies if, after subtraction of background value, optical density (OD 492 nm) of a 1:500 serum dilution was above 0.1. Antibody titers were defined as 'low' (OD_{492} 0.1–1.0), 'medium' (OD_{492} 1.0–2.0), or 'high' ($OD_{492}>2.0$).

2.3. Isolation of leukocytes

Polymorphonuclear granulocytes (PMN) were isolated from heparinized venous blood of healthy volunteers using Ficoll-Histopaque (Sigma, St. Louis, MO) gradient centrifugation. Remaining erythrocytes were removed by hypotonic lysis. PMN were washed in RPMI 1640 medium (Invitrogen, The Netherlands), supplemented with 10% heat-inactivated fetal calf serum, and resuspended to a concentration of 1×10^7 PMN/ml. Cell viability was >99% as determined by Trypan blue exclusion.

2.4. Antibodies

Fluorescein-isothiocyanate (FITC)-conjugated 22 (mouse IgG1, Medarex, Annandale, NJ), IV.3 (mIgG2b, Medarex,

Annandale, NJ), 3G8 (mIgG1, Medarex, Annandale, NJ), and PE-conjugated A59 (mIgG1, BD Biosciences) monoclonal antibodies (mAb) were used to routinely assess surface expression levels of FcγRI (CD64), FcγRII (CD32), FcγRIII (CD16), and FcαRI (CD89), respectively, on PMN before each experiment (Rodriguez et al., 1999, 2001). FcαRI (CD89)-specific mAb 2D11 was used at a concentration of 10 μg/ml for select FcαRI (CD89) blocking experiments (Westerhuis et al., 1999).

2.5. IgA isolation from serum

IgA purification was performed according to methods described previously (van der Boog et al., 2002). Briefly, serum from three GBS patients, containing GM1-specific IgA, and two healthy controls were loaded on anti-IgA affinity columns (HisA 43, kindly provided by Dr. J. van den Born, VU Medical Center, Amsterdam, The Netherlands). Columns were washed with 0.5× PBS and 0.3 M NaCl, before elution of IgA with 0.1 M glycine/0.3 M NaCl (pH 2.8). Directly after elution, the fractions were neutralized with 1 M Tris buffer (pH 8.0). IgA-containing fractions, as assessed by ELISA (de Fijter et al., 1995), were pooled and dialyzed against PBS, containing 2 mM EDTA.

2.6. Degranulation assay

Ninety-six well plates (NUNC, Polysorp®, Roskilde, Denmark) were coated with 0.5 μg GM1, GQ1b or GD1b (all Calbiochem, San Diego, CA, except GD1b: Sigma) in 100 μl of methanol. Next, wells were saturated with PBS containing 1% bovine serum albumin (BSA, Roche Diagnostics, Mannheim, Germany) for 4 h at room temperature. Wells coated with PBS 1% BSA, but without ganglioside, served as control for a specific leukocyte activation, since coating with BSA yielded similar background signals as coating with an irrelevant ganglioside (van Sorge et al., 2003a). Patient sera and sera from healthy controls (lacking significant ganglioside-specific antibody titers as determined by ELISA) were serially diluted in triplicate (starting at 1:20 in PBS 1% BSA) and incubated overnight at 4 °C. In select experiments, purified IgA fractions from patient and control sera were serially diluted in triplicate (starting from 100 μg/ml) and incubated overnight at 4 °C. Plates were washed six times with PBS, prior to addition of 1.5×10^5 freshly-isolated PMN from healthy volunteers in 150 μl Hank's balanced saline solution 1% BSA per well. After incubation for 1 h at 37 °C, plates were centrifuged for 5 min at 1200 rpm. Supernatants were harvested and stored at −80 °C. Lactoferrin concentrations in supernatants were determined by sandwich ELISA (see below). Supernatants from PMN stimulated with formyl-metleuphe (Sigma) in the presence of cytochalasin B (Sigma), and PMN-lysates (after incubation with 1% Triton-X for 5 min) were used as positive controls. For control experiments, wells were coated with dilution series of polyclonal IgG (ranging from 0.5–100 μg/ml) or monomeric IgA (ranging from 0.05–200 μg/ml) in PBS, blocked with BSA, after which PMN were added.

2.7. Lactoferrin sandwich ELISA

Lactoferrin is a marker for specific granule release triggered by leukocyte FcR crosslinking (van Sorge et al., 2003b). Lactoferrin concentrations in harvested supernatants were measured using a lactoferrin sandwich ELISA as described before (van Spriel et al., 2001; Voice and Lachmann, 1997). Six fixed lactoferrin concentrations (Sigma) in triplicate were used to construct standard curves in each ELISA plate. Degranulation was defined as a lactoferrin concentration of at least 1.5 times the background at the highest serum concentration, in addition to a concentration-dependent decrease (van Sorge et al., 2003a). To compare degranulation activity between sera, quantification of degranulation was achieved by estimating 'best-fitting' curves of serial serum dilutions using Graphpad Prism version 4.00 for Windows (Graphpad software, San Diego, CA). The magnitude of the degranulation response was expressed as the ratio of the area under the curve (AUC) of wells with ($AUC_{ganglioside}$) and without ganglioside (AUC_{BSA}). Previous results showed that FcγR-mediated activation of PMN and monocytes by GM1-specific IgG-containing serum samples correlated well (van Sorge et al., 2003a). Since PMN purification is less laborious than monocyte isolation and yields greater amounts of cells, PMN were used for all experiments. Pilot experiments indicated that lactoferrin release by human PMN quantitatively shows donor-to-donor variation (data not shown). Therefore, experiments comparing degranulation responses induced by ganglioside-specific patient sera were performed on the same day, using freshly isolated PMN from a single donor. All serum samples were tested twice and serum samples containing antibodies with similar ganglioside specificities (i.e. GM1, GQ1b, or GD1b) were tested on the same day.

2.8. Statistics

SPSS (Version 12.0.02 for Windows) was used for statistical analysis. Student's two-tailed *t*-test was used to study differences between ganglioside-specific Ig-induced and background levels of degranulation. Mann–Whitney *U*-test was used to analyze differences in leukocyte degranulation between serum samples containing low, medium or high ganglioside-specific IgG titers. χ^2 test was used for non-parametric testing. Statistical significance was accepted at $p < 0.05$.

3. Results

Initial degranulation experiments were performed with 12 serum samples from Dutch patients with GBS or MFS, containing high IgG titers against either GM1 ($n=8$), GQ1b ($n=3$), or GD1b ($n=1$). Concentration-dependent release of lactoferrin was observed with all sera (Fig. 1A–C).

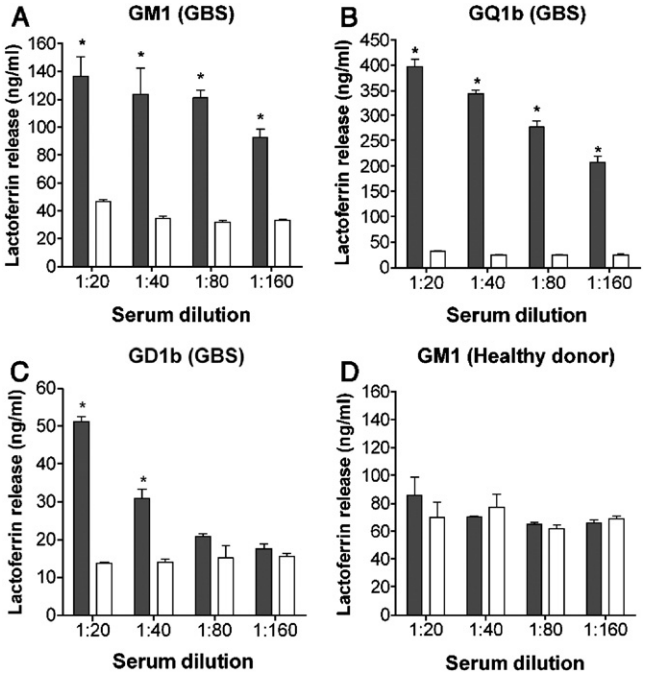

Fig. 1. Ganglioside-specific IgG activates leukocytes, irrespective of antibody specificity. Representative sera from patients with GBS containing (A) GM1, (B) GQ1b and (C) GD1b-specific IgG induce concentration-dependent lactoferrin release by PMN. (D) Healthy donor serum does not induce leukocyte degranulation. Figure D shows representative results from experiments using GM1-coated 96 well plates. Bars are mean ± SD from triplicate. Representative results from one of three independent experiments are shown. Grey bars represent lactoferrin release by PMN in ganglioside-coated wells, white bars lactoferrin release in BSA-coated control wells; *$p<0.05$ (*t*-test).

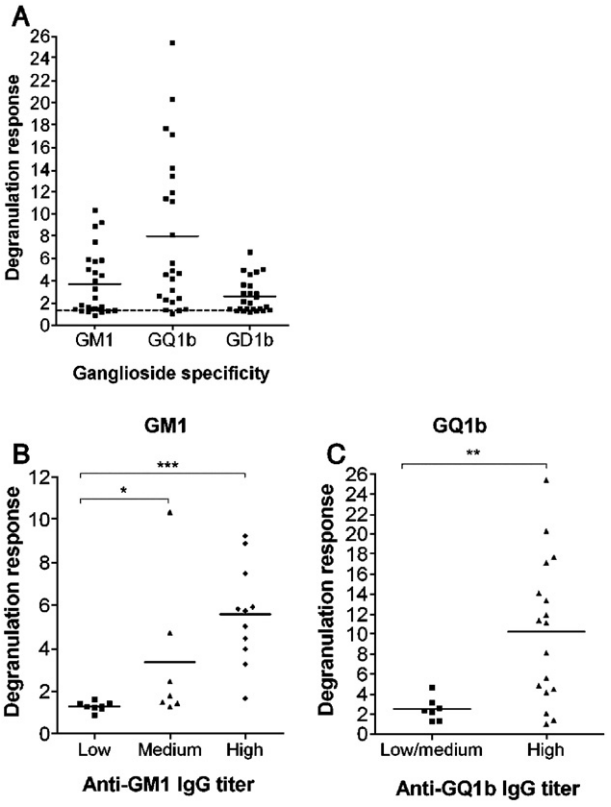

Fig. 2. Magnitude of ganglioside-specific IgG-mediated leukocyte activation and correlation with antibody titer. (A) A panel of 75 patient sera, containing either GM1-specific IgG ($n=26$), GQ1b-specific IgG ($n=24$) or GD1b-specific IgG ($n=25$), were tested for their capacity to activate leukocytes. Quantification of the degranulation response is described in Materials and methods (Section 2.7). Each dot represents an individual serum sample. The dotted line represents background degranulation levels. Mean degranulation is depicted as a line. Leukocyte activation, represented by degranulation responses, is most pronounced using sera with high (B) GM1- and (C) GQ1b-specific IgG titers. Ganglioside-specific IgG titers are defined as low, medium, or high, as described in Section 2.2 of materials and methods. Each dot represents an individual serum sample. Mean degranulation is depicted as a line. *$p<0.05$; **$p<0.01$; ***$p<0.001$ (Mann–Whitney *U*-test).

Degranulation was not observed using sera from healthy control subjects without ganglioside-specific IgG (Fig. 1D). Leukocyte activation was ganglioside-specific, as sera containing high titers of GM1-specific IgG did not induce leukocyte degranulation upon incubation in GQ1b-coated ELISA plates (data not shown). These results indicate that ganglioside-specific IgG generally induces leukocyte activation, irrespective of specificity.

Next, we evaluated whether the magnitude of leukocyte degranulation was associated with the titer of ganglioside-specific antibodies. Therefore, 75 patient sera (Table 1), containing GM1-, GQ1b-, or GD1b-specific IgG, were tested in leukocyte degranulation experiments. Degranulation responses above background were observed in 15 out of 26 (57%) GM1-specific IgG-containing patient sera (Fig. 2A, left column); 20 out of 24 (83%) GQ1b-specific IgG-containing patient sera (Fig. 2A, middle column); and 14 out of 25 (54%) of GD1b-specific IgG-containing patient sera (Fig. 2A, right column). Patient sera containing high titer GM1-and GQ1b-specific IgG, but not GD1b-specific IgG (data not shown), induced significantly higher degranulation responses compared to medium or low titer sera (Fig. 2B, C).

The capacity of GM1-specific IgG-containing serum samples to induce leukocyte degranulation was not associated with relatively mild (Hughes' severity score 1–3) or severe disease (Hughes' severity score 4–5) (data not shown), even after inclusion of 15 GM1-specific serum samples from our previous study (Table 2) (van Sorge et al., 2003a). Furthermore, the magnitude of GM1-specific degranulation responses did not differ between sera from patients with mild and severe disease

Table 2
The capacity of GM1-specific antibodies to activate leukocytes is not associated with mild or severe disease in patients with GBS

		GM1[1]	
		Disease severity	
		Mild	Severe
Leukocyte degranulation	Negative	5 (36%)	9 (64%)
	Positive	9 (33%)	18 (67%)

[1]Includes data from this study and a previously published study ($n=41$ patients) (van Sorge et al., 2003a).

(Fig. 3A). Similarly the magnitude of GQ1b- and GD1b-specific IgG-mediated leukocyte degranulation was not significantly higher among patients with severe disease (data not shown). Sera from patients with GQ1b-specific antibodies were obtained from patients with a different clinical presentation (Table 1). The mean magnitude of leukocyte degranulation induced by GQ1b-specific IgG was higher using serum samples from patients with AO and MFS than from GBS patients (Fig. 3B) and correlated with higher GQ1b-specific IgG titers (Fig. 3C). Ganglioside-specific antibody titers were not associated with disease severity in any of the ganglioside subgoups.

GM1-specific IgG frequently displays cross-reactivity with GD1b (Koga et al., 2001). Seven serum samples from GBS patients containing both significant GM1-and GD1b-specific IgG titers were tested in GM1- and GD1b-specific leukocyte degranulation experiments. The results are summarized in Table 3. Two out of seven serum samples activated leukocytes both in GM1 and GD1b-specific assays; two serum samples were negative in both assays; three samples activated leukocytes either in GM1 or GD1b-specific assays.

Table 3
Degranulation induced by GBS sera containing both GM1- and GD1b-specific IgG antibodies

GM1-specific IgG titer[1]	Degranulation[2]	GD1b-specific IgG titer[1]	Degranulation[2]
High	3.2	Medium	5.1
High	5.3	High	2.9
Low	Background	High	Background
Medium	Background	Low	Background
Low	Background	High	3.5
High	4.1	Low	Background
Medium	2.5	Medium	Background

[1]Antibody titers: 'low' (OD_{492} 0.1–1.0), 'medium' (OD_{492} 1.0–2.0), or 'high' (OD_{492} > 2.0) at a 1:500 serum dilution. [2]Degranulation responses were quantified as described in Material and methods. Values below 1.5 reflect background levels.

Finally, we evaluated the capacity of GM1-specific IgA to activate leukocytes through FcαRI (CD89). Leukocyte degranulation was detectable in wells coated with IgA at concentrations as low as 0.4 μg/ml (Fig. 4A). Next, IgA was purified from serum samples of three patients with GBS, containing GM1-

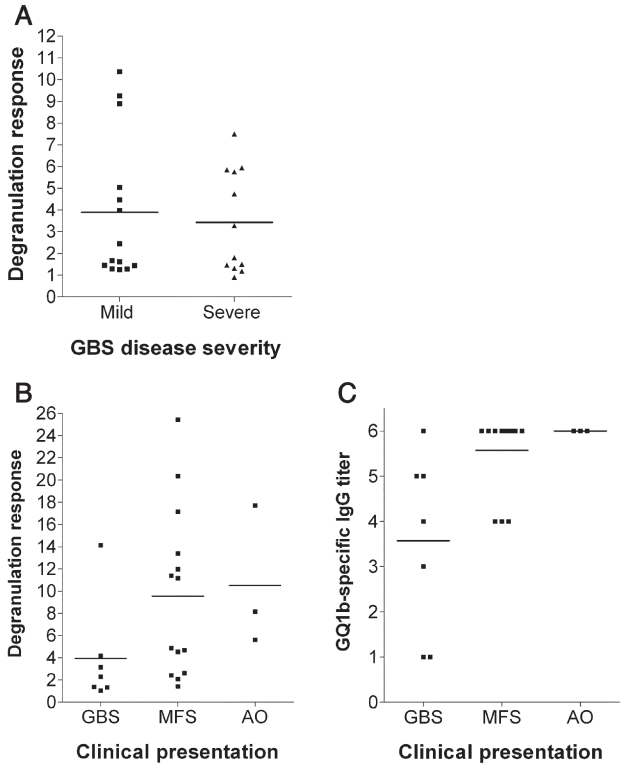

Fig. 3. Correlation between magnitude of ganglioside-specific IgG-mediated leukocyte activation, disease severity and clinical presentation. (A) The magnitude of GM1-specific IgG-mediated leukocyte degranulation is not associated with disease severity. Relatively mild disease is defined as Hughes' scores 1 to 3, severe disease as scores 4 or 5. Lines represent means of degranulation responses. Each dot represents an individual serum sample. (B) The magnitude of GQ1b-specific leukocyte degranulation and (C) GQ1b-specific IgG titers are higher using sera from patients with MFS and acute ophthalmoparesis (AO) as compared to patients with GBS. Lines represent mean values of titer and degranulation responses, respectively. Each dot represents an individual serum sample.

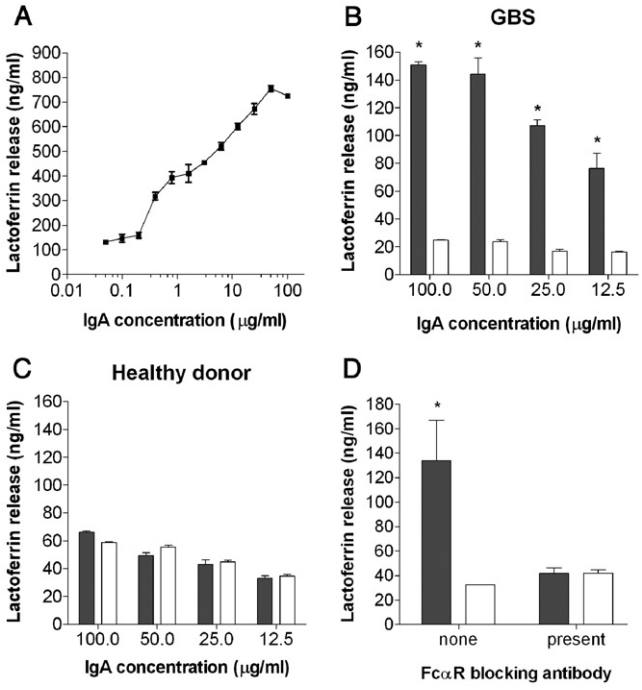

Fig. 4. GM1-specific IgA recruits leukocyte effector functions through FcαRI (CD89) (A) Polyclonal monomeric IgA coated on 96 well plates induces concentration-dependent lactoferrin release from PMN *in vitro*. (B) GM1-specific IgA from GBS patient serum, but not (C) IgA from healthy donor serum, efficiently activates PMN, as indicated by lactoferrin release. IgA fractions were isolated from sera of three patients with GBS and two healthy controls. Experiments were performed three times in triplicate using PMN from different donors. Representative results from one experiment are shown. Grey bars represent lactoferrin release by PMN in ganglioside-coated wells, white bars lactoferrin release in BSA-coated control wells. (D) Lactoferrin release induced by GM1-specific IgA is abrogated by FcαRI-specific blocking mAb (2D11, 10 μg/ml). Grey bars represent lactoferrin release by PMN in GM1-coated wells, white bars lactoferrin release in BSA-coated control wells. *$p < 0.05$ (*t*-test).

specific IgA as determined by ELISA, and from serum samples of two healthy controls without GM1-specific IgA, and used in degranulation experiments. Purified IgA from GBS patients induced leukocyte degranulation (Fig. 4B), whereas such activity was not observed using IgA from control subjects (Fig. 4C). The presence of a specific FcαRI (CD89)-blocking mAb abrogated IgA-induced degranulation, indicating that this function is FcαRI (CD89)-dependent (Fig. 4D).

4. Discussion

Lymphocyte and macrophage infiltrates are detected in nerves from patients with acute inflammatory demyelinating polyneuropathy (Hafer-Macko et al., 1996b). In nerves from patients with acute motor axonal neuropathy (AMAN), macrophages are detected at the nodes of Ranvier and in the periaxonal space (Griffin et al., 1996; Hafer-Macko et al., 1996a). FcγR and FcαRI (CD89) are highly expressed on macrophages, and link the specificity of ganglioside-specific antibodies to potent leukocyte effector functions. Hence, activation of inflammatory cells through antibody receptors could contribute to inflammation in nerves and nerve roots. We previously showed that GM1-specific IgG activates leukocytes via FcγR (van Sorge et al., 2003a). Moreover, leukocyte-activating properties of GM1-specific antibodies are strongly associated with the occurrence of flaccid paralysis in a rabbit model for AMAN (van Sorge et al., in press). Interestingly, intravenous IgG (IVIg) treatment for GBS is thought to exert its beneficial effect at least partially via blocking of activating FcγR (Dalakas, 1998). These observations emphasize the relevance of studying leukocyte activation by ganglioside-specific antibodies in serum from patients with GBS or MFS. The data presented here show that not only GM1-specific IgG (van Sorge et al., 2003a), but also GD1b-, and GQ1b-specific IgG in serum from patients with GBS, MFS, and AO activate leukocytes, as measured by their capacity to induce degranulation of PMN. In addition to ganglioside-specific IgG, IgA may contribute to the recruitment of leukocyte effector functions via FcαRI (CD89).

Although the role of PMN in the pathogenesis of GBS and MFS is unknown, PMN were used as effector cells, since they abundantly express FcγR and can be readily harvested in large numbers. Furthermore, activation of granulocytes and monocytes by ganglioside-specific antibodies showed a high degree of correlation in previous experiments (van Sorge et al., 2003a). In addition, FcγRIIa, the most important FcγR subclass on PMN for triggering degranulation (van Sorge et al., 2003a), is constitutively expressed on PMN, monocytes and macrophages.

A panel of 75 patient sera was evaluated for their capacity to activate inflammatory cells. Overall, 49 out of 75 (65%) tested sera induced leukocyte degranulation. In our previous study, leukocyte activation was only observed in sera with relatively high GM1-specific IgG titers (van Sorge et al., 2003a). Correspondingly, the majority of sera that failed to activate leukocytes in the present study displayed relatively low ganglioside-specific IgG titers. The GQ1b subgroup contained the highest percentage of serum samples capable of activating leukocytes, which is likely explained by the high percentage of high titer sera in this group (71% in GQ1b group vs. 42% in GM1 and 52% in GD1b groups). Similarly, GQ1b-IgG titers and GQ1b-specific leukocyte activation were both higher using sera from patients with MFS and AO (82%) as compared to sera from patients with GBS (43%). These data suggest that ganglioside-specific IgG can recruit leukocyte effector functions in addition to complement activation (Halstead et al., 2005, 2004; Zhang et al., 2004), irrespective of ganglioside specificity of the antibodies.

Although the magnitude of the degranulation response was generally higher using sera with high as compared to sera with medium or low ganglioside-specific IgG titers, several serum samples with high specific IgG titers failed to activate leukocytes, whereas several serum samples with low to medium titers activated leukocytes efficiently. This indicates that titer is not the only characteristic that predicts biological activity of ganglioside-specific antibodies. Antibody avidity is an important pathogenic characteristic of autoantibodies (Lopez et al., 2002; Mitzutamari et al., 1998), as this property correlates with antibody functionality (Hetherington and Lepow, 1992; Romero-Steiner et al., 1999). Avidity of experimentally elicited GM1-specific IgG from rabbits without signs of neuropathy was significantly lower than similar antibodies in sera from patients and experimental animals with flaccid paralysis (Comín et al., 2006; Lopez et al., 2002). Correspondingly, we observed that GM1-specific IgG from rabbits with experimentally-induced neuropathy activated complement and leukocyte degranulation, in contrast to GM1-specific serum samples from rabbits without neurological deficits (van Sorge et al., in press). Affinity maturation and antibody class switch (i.e. the appearance of IgG and IgA) may endow naturally occurring ganglioside-specific IgM antibodies (Alaniz et al., 2004) with pathogenic, i.e. leukocyte or complement-activating, properties.

The association of GM1-specific IgG1, but not IgG3, with poor long term outcome (Koga et al., 2003) may suggest differences in recruitment of inflammatory effector functions. However, major differences in degranulation efficacy between sera containing solely GM1-specific IgG1 or IgG3 were not observed in a limited number of pilot experiments.

The magnitude of leukocyte degranulation induced by GM1-specific IgG was not significantly higher in patients with severe GBS. Similarly, no association between leukocyte degranulation induced by GQ1b- or GD1b-specific antibodies with disease severity was found (data not shown). It should be noted that the Hughes' severity scale may not accurately reflect disease severity in these latter patient groups, since GQ1b and GD1b antibody specificities are associated with other clinical deficits than weakness. Furthermore, severity may not represent the best clinical correlate for the vigor of the inflammatory response. Possibly, the extent of axonal damage and outcome are

determined by the sum of complement and leukocyte-mediated damage to peripheral nerves.

Ganglioside-specific IgG often displays cross-reactivity with other gangliosides. GM1-specific IgG for example, cross-reacts with asialo-GM1, GM1b, GalNAc-GD1a, and GD1b (Koga et al., 2001). The presence of monospecific GD1b-IgG in serum from patients with GBS is associated with pronounced ataxia in the absence of weakness (Pan et al., 2001; Wicklein et al., 1997). Similar findings were also reported in GD1b-sensitized rabbits (Kusunoki et al., 1999). Our data suggest that biological activity of GD1b-specific IgG is not confined to monospecific antibodies in serum from patients with ataxic GBS. Serum samples containing GD1b-specific IgG obtained from patients with ($n=1$) or without pronounced ataxia (13 out of 24) both activated leukocytes efficiently. In addition, two sera containing IgG reacting with GM1 and GD1b activated leukocytes in either assay. This may imply that the biological activity of cross-reactive antibodies is not confined to one of its specificities.

In addition to IgG, GM1-specific IgA activated leukocytes. The presence of ganglioside-specific IgA is associated with poor outcome in patients with GBS and MFS (Ilyas et al., 1992; Koga et al., 1998), but its inflammatory capacity has not been studied in detail. IgA is traditionally regarded as an "anti-inflammatory" isotype, inhibiting adhesion of microorganisms at mucosal sites, rather than triggering inflammation (van Egmond et al., 2001). This concept has been challenged by the observation that activation of leukocyte FcαRI (CD89), which is expressed on PMN, monocytes and macrophages, triggers a plethora of pro-inflammatory leukocyte effector functions, similar to IgG receptors (van Egmond et al., 2001; van Sorge et al., 2003b). Although FcαRI (CD89) expression is lower than FcγR expression on leukocytes, expression is quickly upregulated (Shen et al., 1994). Importantly, FcαRI (CD89) triggers leukocyte effector functions at least as effectively as FcγR (Stockmeyer et al., 2000; van der Pol et al., 2000). Engagement of FcαRI (CD89) by ganglioside-specific IgA, in addition to the possibility of complement activation via the lectin pathway (Roos et al., 2001), may provide an explanation for the association between GM1-specific IgA and poor outcome. Theoretically, simultaneous engagement of FcαRI (CD89) and FcγR may enhance inflammatory activity of leukocytes (Stewart and Kerr, 1991).

The relative contribution of leukocytes and complement in GBS pathogenesis remains to be determined. Several recent studies documented that GBS-associated nerve damage may result from ganglioside-specific IgM-, and IgG-induced complement activation (Goodyear et al., 1999; Halstead et al., 2004; Zhang et al., 2004). The present data show that both ganglioside-specific IgG and IgA antibodies in serum from GBS patients generally activate leukocytes, which may contribute to GBS pathogenesis.

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