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Multifocal motor neuropathy is not associated with altered innate immune responses to endotoxin

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ABSTRACT

Objective: Antibody- and complement-mediated peripheral nerve inflammation are central in the pathogenesis of MMN. Here, we studied innate immune responses to endotoxin in patients with MMN and controls to further our understanding of MMN risk factors and disease modifiers.
Methods: We stimulated whole blood of 52 patients with MMN and 24 controls with endotoxin and collected plasma. With a multiplex assay, we determined levels of the immunoregulating proteins IL-1RA, IL-1β, IL-6, IL-10, IL-21, TNF-α, IL-8 and CD40L in unstimulated and LPS-stimulated plasma. We compared baseline and stimulated protein levels between patients and controls and correlated concentrations to clinical parameters.
Results: Protein level changes after stimulation were comparable between groups ($p > 0.05$). IL-1RA, IL-1β, IL-6 and IL-21 baseline concentrations showed a positive correlation with monthly IVIg dosage (all corrected p-values < 0.016). Patients with anti-GM1 IgM antibodies showed a more pronounced IL-21 increase after stimulation (p 0.048).
Conclusions: Altered endotoxin-induced innate immune responses are unlikely to be a susceptibility factor for MMN.

1. Introduction

Multifocal motor neuropathy (MMN) is a rare, asymmetric, chronic and immune-mediated neuropathy. It initially causes distal asymmetrical muscle weakness, which can be reversed partially with regular infusions of intravenously or subcutaneously administered immunoglobulins (IVIg, scIg) [1–4]. Despite treatment, accumulating motor nerve axonal damage will eventually lead to refractory deficits, causing permanent and significant disability in about 20% of patients [5,6]. MMN is characterized by the presence of IgM antibodies to paranodal components, primarily the ganglioside GM1 [5,7,8]. Upon binding, these antibodies can trigger damage to axons or myelin through activation of the classical pathway of complement [1,7,9–11]. The origin of pathogenic antibodies in MMN is unknown. MMN is not characterized by systemic inflammation as shown by the lack of inflammatory markers in peripheral blood and cerebrospinal fluid [12,13]. In patients with Guillain-Barré syndrome (GBS), an acute inflammatory neuropathy associated with preceding infections that is characterized by the presence of anti-ganglioside IgG autoantibodies, increased TLR4-mediated innate immune responses were shown to be a critical host susceptibility factor [14]. In this study, we aimed to explore the possibility of an altered innate immune response as a susceptibility factor for MMN. To this end, we stimulated blood of patients with MMN and controls with lipopolysaccharide (LPS), a potent endotoxin known to induce an innate immune response after binding to TLR4 [15]. We compared levels of secreted immunoregulatory proteins and studied associations with clinical parameters.

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2. Methods

2.1. Study population

We enrolled patients with MMN as part of a cross-sectional study in the Netherlands (MAIN study) [2]. All patients had been diagnosed at the outpatient clinic of the University Medical Center Utrecht (UMCU), a Dutch national center for MMN and other neuromuscular disorders. All patients fulfilled the most recent 2010 diagnostic criteria for definite, probable or possible MMN and all patients were Dutch [16]. Control subjects were enrolled through the Prospective ALS study in The Netherlands (PAN study), a prospective Dutch population-based case-control study performed at the UMCU [17,18]. Additionally, we obtained blood samples from healthy control subjects via the voluntary in-house donor facility of the UMC Utrecht.

2.2. Clinical data

We obtained routine clinical and demographic data from our MMN database; when necessary, these were supplemented with data obtained from the UMCU patient files [2]. We recorded sex, age at onset, age at diagnosis and diagnostic delay, anti-GM1 IgM antibody status, monthly IVIg dosage and muscle strength, displayed as an MRC sum score (see below), for all patients at the time of inclusion.

We defined onset of MMN as the first patient-reported complaint of weakness in arm or leg muscles and diagnostic delay as the time that lapsed between onset and MMN diagnosis. We used a standardized ELISA to document the presence of anti-GM1 IgM antibodies in serum as described previously [19,20]. We calculated the monthly IVIg dosage at the time of inclusion. On the day of inclusion in the MAIN study, we performed a standardized neurological examination that included testing muscle strength, using the 6-point Medical Research Council (MRC) scale, which ranges from 0 (no muscle contraction) to 5 (normal muscle strength against resistance). We documented MRC scores for left and right shoulder abduction, elbow flexion and extension, wrist flexion and extension, finger flexion and extension, finger spreading, thumb adduction, abduction and flexion, hip flexion, knee flexion and extension, foot flexion and extension, and toe flexion and extension and calculated the MRC sum score (range 0–180) [2].

2.3. LPS stimulation experiment

Upon inclusion in the 2015 MAIN study, we collected 10 mL of blood in sodium-heparin vacutainers by venipuncture and used it immediately for experimental procedures. For baseline concentrations, we centrifuged 1.5 mL of unstimulated blood at 1500 x *g* for 10 min at room temperature, collected plasma and stored it immediately at -80^{0}C until further analysis. For whole blood stimulation experiments, we used 24-well plates. Per subject, we filled one well with 1 mL of freshly drawn whole blood and added LPS at a final concentration of 1 μg/mL (LPS from *Escherichia coli* O127:B8, Sigma L3129, 5 mg/mL in millQ (MQ) water). We filled a second well per subject with 1 mL of whole blood and 5 μL of MQ, which served as a control. We incubated plates at 37^{0}C for 4 h in an incubator with 5% CO_2, followed by centrifugation at 1500 x*g* for 10 min at room temperature. We collected plasma and stored samples at -80 °C until further analysis.

2.4. Luminex assay

We used previously validated multiplex immunoassays (Luminex platform) to determine plasma protein levels [21]. All assays were performed at the ISO-certified multiplex core facility of the UMCU. We determined levels of the immunoregulating proteins IL-1RA, IL-1β, IL-6, IL-10, IL-21, TNF-α, IL-8 and CD40L in plasma of unstimulated, MQ-, and LPS-stimulated whole blood samples.

We considered data that were out of the detection range of the Luminex platform (out of range, OOR), or samples that were extrapolated in the lower or upper ranges from the assay sensitivity to have been measured imprecisely and therefore replaced these values using the following method [22]. Low OOR or extrapolated values in the low range were replaced by either the lower limit of quantification (LLOQ) divided by 2, or, when the lowest measured value of the marker in question was lower than the LLOQ, by the lowest value divided by 2. OOR or extrapolated values at the upper range were, similarly, replaced by the upper limit of quantification, or the highest measured value times 2.

2.5. Statistical analysis

For statistical analyses, we used R (version 3.6.1). First, we calculated an LPS-induced change in protein levels by subtracting the change from baseline in the control (MQ) condition from the change from baseline in the LPS stimulation experiment. We compared the LPS-induced change between patients and controls using a Mann-Whitney *U* test. Second, we correlated clinical parameters to baseline protein levels and their LPS-induced change. We assessed correlations between the baseline protein levels and the LPS-induced change with monthly IVIg dosage, disease duration and MRC sum score using Spearman's correlation coefficient. We compared results between patients with and without anti-GM1 IgM antibodies using the Mann-Whitney U test. Because of sample size assumptions, all analyses of continuous variables, as described above, were only performed for proteins whose above or below OOR outcomes comprised <50% of the total data points. If this was not the case, we compared the percentage of OOR and extrapolated data points between patients and controls using a χ^2 test.

To assess differential clustering of data, we performed a principal component analysis (PCA) using baseline concentrations and concentration changes after stimulation with LPS separately. The in-built PCA function of R was used. Ellipses showing the 95% confidence intervals of the t-distribution were drawn per subgroup.

All *p*-values were adjusted for multiple testing using the Bonferroni method.

2.6. Standard protocol approvals, registrations, and patient consents

The locally appointed ethic committee of the University Medical Center Utrecht approved this study (METC Utrecht, METC.NL.041). All included subjects gave written informed consent prior to inclusion in this study.

2.7. Data availability statement

The data that support the findings in this study will be available on reasonable request from the corresponding author.

3. Results

3.1. Study population

We included 52 patients with MMN and 24 control subjects (12 through the PAN study and 12 via the UMCU in-house voluntary healthy donor service). The Luminex assay showed normal standard negative control levels in 47/52 (90%) of the MMN samples and in 24/24 (100%) of the control samples. Baseline characteristics are shown in Table 1. IVIg dosage (median 52 g per month) was positively skewed, ranging from 20 to 212 g per month. Seven patients with MMN (15%) were not treated with IVIg at the moment of inclusion in this study.

3.2. Detection limits

The Luminex platform used for our analyses has a large sensitivity range. However, as detection limits differed per protein included in our

Table 1
Baseline characteristics of patients with MMN and controls.

	MMN ($n = 47$)	Controls ($n = 24$)	
		PAN study ($n = 12$)	UMCU (n = 12)
Male sex (n, %)	36 (77)	10 (83)	NA
Age at inclusion (years)[$]	58 (17)	70 (10)	NA
Autoimmune disease (n, %)	3/43 (7)	0/12 (0)	
Vitiligo	1/43 (2)	0 (0)	
Astmatic bronchitis	2/43 (5)	0 (0)	
Immunosuppressant therapy (n, %)	0/47 (0)	0/12 (0)	
Disease duration at inclusion (years)[$]	16 (13)		
Age at onset (years)[$]	43 (11)		
Age at diagnosis (years)[$]	48 (8)		
Diagnostic delay (months)[$]	32 (46)		
EFNS MMN diagnosis (n/N, %)			
Definite MMN	32/45 (71)		
Probable MMN	12/45 (27)		
Possible MMN	1/45 (2)		
Anti-GM1 IgM positive (n/N, %)	22/42 (54)		
IVIg treatment at inclusion (n/N, %)	40/47 (85)		
IVIg dosage at inclusion (gr/month)[$]	52 (49)		
MRC sum score at inclusion[$]	168 (11)		

MMN = multifocal motor neuropathy, MRC = medical research council, NA = not applicable, PAN = prospective ALS study in the Netherlands, UMCU = University Medical Center Utrecht.
[$] Values displayed as median (IQR).

analyses, the reliability of the measurements of each protein might also differ. We have, therefore, provided an overview of all out of range (OOR) data in Table 2. Baseline concentrations of IL-1RA, IL-1β and IL-6 were more often out of range in control subjects than in patients with MMN (χ^2 test, p-values all <0.001). Baseline levels of IL-10 and TNF-α were below the detection limit in >50% of the subjects, in both the control and MMN group.

OOR data after LPS stimulation were evenly distributed between controls and MMN patients for all proteins. IL-6 and TNF-α data were above detection limits in >50% of the subjects in both groups. Two subjects (one patient and one control) had CD40L levels below the detection limit, at baseline as well as after LPS stimulation.

As sufficient within-range data were available, we decided to compare baseline levels of IL-21, IL-8 and CD40L, and LPS-induced changes of IL-1RA, IL-1β, IL-10, IL-21, IL-8 and CD40L, between control subjects and patients with MMN.

3.3. Protein concentrations do not correlate with age and sex

We determined the correlation between baseline protein concentrations and the LPS-induced changes to age at inclusion and sex in all subjects for whom these variables were known ($n = 64$; sex and age at inclusion of 12 control subjects included via the voluntary in-house UMCU donor facility were not known). We found no correlations between protein levels at baseline or after stimulation with age (Spearman correlations, corrected $p > 0.27$ in all comparisons, data not shown) or sex (Mann-Whitney U tests, corrected p-values 1.00 in all comparisons, data not shown). It was, therefore, possible to include in our further analyses the data of 12 control subjects for whom sex and age at inclusion were not known.

3.4. Comparison of protein concentrations between patients with MMN and controls

Baseline and LPS-stimulated protein concentrations of IL-1RA, IL-1β, IL-10, IL-21, IL-8 and CD40L are shown in Table 3. Baseline IL-21 and IL-8 concentrations were higher in patients with MMN than in controls (Mann-Whitney U test, $p < 0.001$ in both comparisons). Baseline CD40L concentration did not differ between groups (Mann-Whitney U test, p 0.62).

LPS-induced protein concentration changes did not differ between patients with MMN and controls (Mann-Whitney U test, p-values all >0.05). Interestingly, in both patients and controls, we observed a decrease in IL-21 levels after LPS stimulation in some subjects, and no or only a moderate increase in others (IL-21 fold change range 0.81–1.51). As only one subject had a change exceeding 1.5-fold, we conclude that, at a group level, IL-21 increase did not occur consistently within four hours of LPS stimulation.

Table 2
Overview of Out of Range (OOR) data, separately shown for patients with MMN and controls, as determined in plasma obtained at baseline and after stimulation with MQ and with LPS. Uncorrected Chi-squared or Fisher's test p-values are shown.

Protein	Baseline ($t = 0$ h)				MQ stimulation ($t = 4$ h)				LPS stimulation (t = 4 h)			
	< OOR	MMN	Controls	p-value	< OOR	MMN	Controls	p-value	> OOR	MMN	Controls	p-value
IL-1RA	19 (27)	3 (6)	16 (67)	<0.001*	9 (13)	2 (4)	7 (29)	0.009*	1 (1)	1 (2)	0 (0)	1.00
IL-1β	42 (59)	19 (40)	23 (96)	<0.001*	42 (59)	20 (43)	22 (92)	<0.001*	27 (38)	18 (38)	9 (38)	1.00
IL-6	23 (32)	5 (11)	18 (75)	<0.001*	9 (13)	1 (2)	8 (33)	<0.001*	57 (80)	37 (79)	20 (83)	0.76
IL-10	41 (58)	26 (55)	15 (63)	0.74	42 (59)	26 (55)	16 (67)	0.51	0 (0)	0 (0)	0 (0)	1.00
IL-21	0 (0)	0 (0)	0 (0)	1.00	0 (0)	0 (0)	0 (0)	1.00	0 (0)	0 (0)	0 (0)	1.00
TNF-α	55 (77)	34 (72)	21 (88)	0.25	12 (17)	9 (19)	3 (13)	0.74	45 (63)	29 (62)	16 (67)	0.88
IL-8	0 (0)	0 (0)	0 (0)	1.00	0 (0)	0 (0)	0 (0)	1.00	10 (14)	4 (9)	6 (25)	0.077
CD40L	2	1 (2)	1 (4)	1.00	2 (3)	1 (2)	1 (4)	1.00	0 (0)	0 (0)	0 (0)	1.00

All values are depicted as n (%).
[*] Statistically significant after Bonferroni p-value adjustment.

Table 3
Baseline (T0) concentrations, concentrations after stimulation with LPS ($T = 4$ h) and concentration increase (Δ) of IL-1RA, IL-1β, IL-6, IL-10, IL-21, TNF-α, IL-8 and CD40L in patients with MMN and controls. All concentrations were measured in pg/mL. The uncorrected Mann-Whitney *U* test *p*-value is shown for comparisons where <50% of data points were measured out of the detection limits in both groups.

Protein	MMN			Controls				
	T0	T = 4 h	Δ	T0	T = 4 h	Δ	*p* T0	*p* Δ
IL-1RA	353 (210)	10,009 (6225)	9557 (6168)	4.20 (186)	8866 (3526)	8862 (3503)	–	0.73
IL-1β	3.04 (4.45)	3142 (9223)	3138 (9223)	0.105 (0)	3192 (9115)	3188 (9115)	–	0.69
IL-6	34.8 (40.3)	23,559 (106)	23,526 (116)	1.40 (0.865)	23,559 (13)	23,557 (18)	–	–
IL-10	0.965 (2.45)	62.3 (52.6)	57.4 (51.5)	0.965 (2.80)	78.7 (67.4)	75.6 (65.7)	–	0.14
IL-21	460 (227)	461 (227)	2.49 (72.4)	273 (36.3)	277 (52.9)	4.60 (31.2)	<0.001*	0.86
TNF-α	0.250 (0.780)	21,778 (17291)	21,777 (17292)	0.250 (0)	21,778 (16699)	21,777 (16699)	–	–
IL-8	35.2 (25.2)	3777 (2175)	3757 (2191)	11.6 (5.36)	4530 (11596)	4502 (11596)	<0.001*	0.08
CD40L	137 (201)	506 (433)	203 (275)	262 (109)	530 (706)	186 (452)	0.62	0.86

All values are depicted as median (IQR).
* Statistically significant after Bonferroni p-value adjustment.

3.5. Correlation of immunoregulating protein levels with patient characteristics

3.5.1. IVIg therapy

We correlated baseline protein concentrations and associated LPS-induced changes to the monthly IVIg dosage and found a positive and statistically significant correlation with baseline concentrations of IL-1RA, IL-1β, IL-6, and IL-21 (Fig. 1).

We hypothesized that IVIg dosage might be a proxy for disease severity, where patients with more severe MMN would receive a higher IVIg dosage. However, when we correlated IL-1RA, IL-1β, IL-6 and IL-21 baseline concentrations to disease duration and the MRC sum score at inclusion, we did not observe significant associations (Spearman correlation, $p > 0.05$ in all correlations, data not shown). Moreover, none of the proteins' LPS-induced change was correlated with monthly IVIg dosage (Fig. 1).

Next, to further study the association between IVIg treatment and protein concentrations at baseline and after LPS stimulation, we compared protein concentrations between controls and patients with MMN who were treatment-naïve at the time of inclusion in this study ($n = 7$). Baseline IL-8 concentration was higher in treatment-naïve patients than in controls (Mann-Whitney *U* test, *p* 0.0049), but none of the other proteins' concentrations, either at baseline or after LPS stimulation, differed between groups (Mann-Whitney U test, unadjusted *p*-values all >0.05, data not shown).

Next, as baseline concentrations of IL-8 and IL-21 were higher in MMN patients, but were also associated with IVIg dosage, we corrected these for monthly IVIg dosage using a linear regression analysis, including all subjects. We found that the difference in IL-21 baseline concentration was dependent on IVIg dosage only (monthly IVIg dosage: beta = 2.55, $t = 3.17$, *p* 0.002; subject group: beta = 81.7, $t = 1.21$, *p* 0.229), whereas baseline IL-8 concentrations remained higher in MMN patients, also after correcting for IVIg dosage (monthly IVIg dosage: beta = 0.20, $t = 2.46$, *p* 0.016; subject group: beta = 16.1, $t = 2.36$, *p* 0.021).

In summary, these results indicate that patients with MMN have higher baseline IL-8 concentrations than controls, independent of IVIg therapy or dosage. However, the observed increased baseline concentrations of IL-1RA, IL-1β, IL-6 and IL-21 in patients may be a consequence of IVIg therapy, rather than a reflection of the immunopathology underlying MMN. Finally, the protein concentration changes after LPS stimulation prove to be independent of IVIg therapy.

3.5.2. Anti-GM1 IgM antibody status

Since patients with anti-GM1 antibodies might have a different immunopathology underlying their disease than patients without these antibodies, we studied possible differences in the innate immune response by comparing baseline concentrations and LPS-induced changes between patients with and without anti-GM1 IgM antibodies (Fig. 2). Baseline concentrations of IL-1RA were increased in anti-GM1 IgM positive patients (Mann-Whitney *U* test, corrected *p* 0.030). IL-1β and IL-21 showed a similar trend. When corrected for monthly IVIg dosage using linear regression analysis, we found that the difference between groups depended on monthly IVIg dosage only (monthly IVIg dosage: beta = 2.1, $t = 2.627$, *p* 0.012; anti-GM1 IgM antibody status: beta = 95.3, $t = 1.829$, *p* 0.075). Therefore, baseline concentrations of all analytes did not differ between patients with or without anti-GM1 IgM antibodies.

When comparing protein concentrations between anti-GM1 IgM antibody groups after LPS stimulation, we did not identify differences in the increase of IL-1RA, IL-1β, IL-10, IL-8 and CD40L (Fig. 2). Although we concluded that at a group level unequivocal IL-21 stimulation (i.e. at least a 1.5-fold concentration increase after LPS stimulation) did not occur, we observed a concentration increase in patients with anti-GM1 IgM antibodies more often than in patients without anti-GM1 IgM antibodies. The absolute increase after LPS stimulation was higher in patients with anti-GM1 IgM antibodies (Mann-Whitney *U* test, *p* 0.048). The percentage of patients that showed an increase of at least 10% from baseline was also higher in patients with anti-GM1 IgM antibodies than in patients without (8/22 (36%) vs. 1/19 (5%), χ^2 test, *p* 0.016).

3.6. Integrated comparisons using PCA reveals no clustering of data

Since the analyses described above focused on each protein separately, we decided to use a combined approach to study possible clustering of data by performing a principal component analysis (PCA, Fig. 3). For baseline concentrations, controls showed less scattering of data than patients, in line with data presented in Tables 1 and 2, but no unequivocal clustering occurred. After LPS stimulation, we observed no clustering of data when comparing patients and controls. Likewise, no differential clustering was found in either comparison for patients with or without IVIg treatment, or for patients with or without anti-GM1 IgM antibodies.

In conclusion, in addition to comparing each protein separately, no immunological signatures were found for patients with MMN, patients treated with IVIg or patients with anti-GM1 IgM antibodies in this combined approach using PCA.

4. Discussion

Our understanding of the role of the innate immune system in the immunobiology underlying multifocal motor neuropathy is limited. Therefore, we have explored innate immune responses in whole blood samples from patients with MMN and controls following stimulation with the endotoxin LPS. At baseline, patients with MMN had higher IL-8 concentrations than controls. After endotoxin activation, the levels of a range of immunoregulating proteins increased, but these increases did not differ between patients and controls. Hence, our results do not

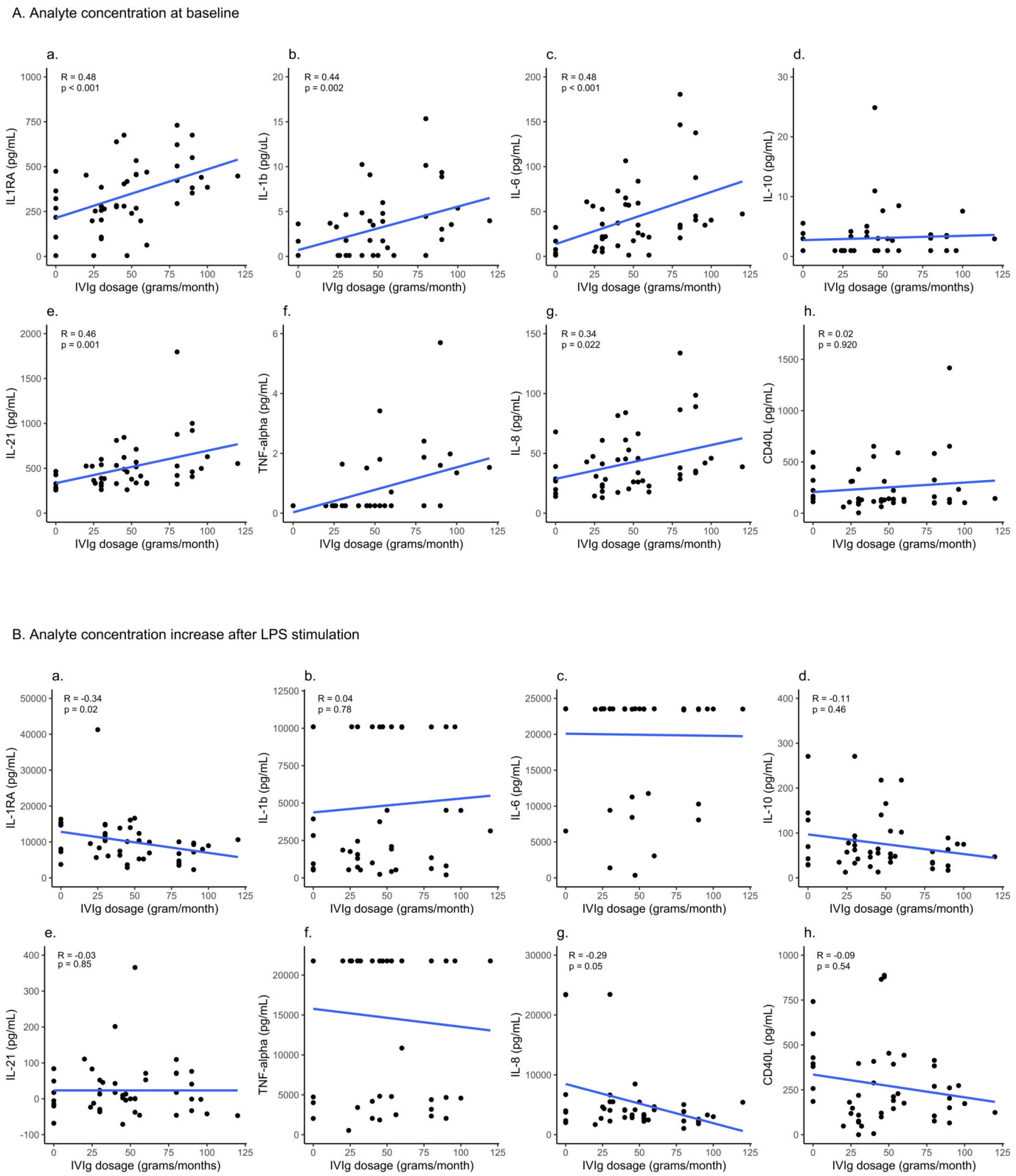

Fig. 1. IVIg dosage correlated to baseline concentrations (panel A) and concentration changes after LPS stimulation (panel B), of IL-1RA, IL-1β, IL-6, IL-10, IL-21, TNF-α, IL-8 and CD40L (plots a-h in both panels). In the upper left corner, Spearman's rho correlation coefficient and its uncorrected p-value are shown for proteins for which >50% of the data points were measured within detection limits.

support variation in the innate immune response as a susceptibility factor for MMN.

Previous studies have shown the value of ex vivo stimulation experiments using endotoxins in uncovering altered innate immune responses and their association with susceptibility to various infectious, autoinflammatory and autoimmune diseases [23–27]. Interestingly, altered innate immune responses have been shown to be a critical host susceptibility factor underlying the loss of immunotolerance to anti-

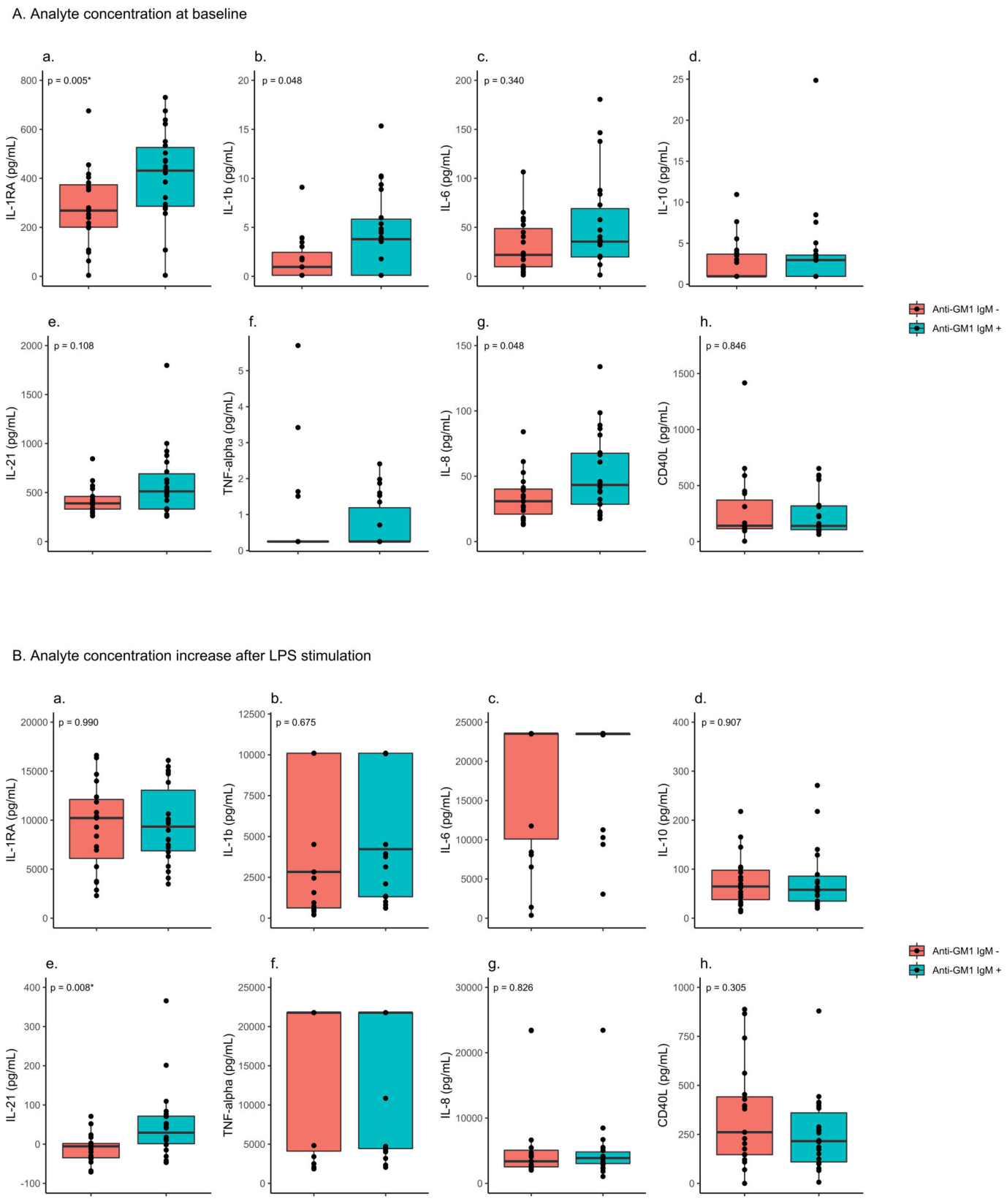

Fig. 2. Baseline concentrations (panel A) and concentration changes after LPS stimulation (panel B) of IL-1RA, IL-1β, IL-6, IL-10, IL-21, TNF-α, IL-8 and CD40L (plots a-h in both panels), stratified by anti-GM1 IgM antibody status in patients with MMN. In the upper left corner, the Mann-Whitney U test's uncorrected p-value is shown for proteins for which at least 50% of the data points were measured within detection limits in both groups.

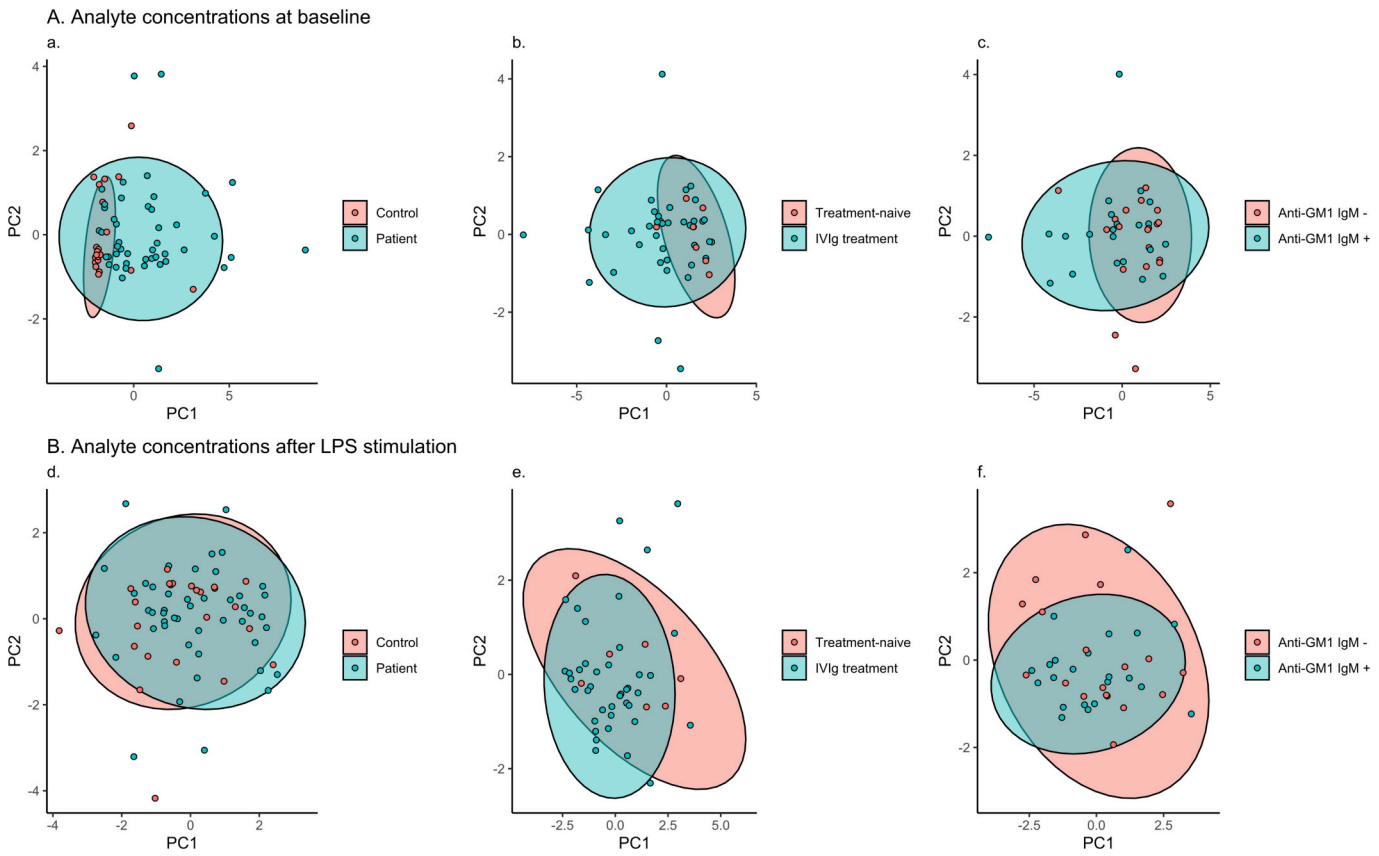

Fig. 3. Principal component analysis (PCA) for baseline concentrations (panel A) and concentration changes after stimulation with LPS (panel B), stratified for patients and controls (plots a. and d.), IVIg treatment status (plots b. and e.) and anti-GM1 IgM antibody status (c. and f.). Data for IL-1RA, IL-1β, IL-6, IL-10, IL-21, TNF-α, IL-8 and CD40L were included in all PCA's.

ganglioside antibodies in Guillain-Barré syndrome (GBS), an acute, monophasic autoimmune neuropathy, characterized by IgG antibodies to gangliosides, including GM1 [28,29]. In GBS, sialylation of *Campylobacter jejuni* endotoxin augments the TLR4-mediated innate immune response by dendritic cells, leading to increased T-cell independent B-cell stimulation [15]. Moreover, this study showed that the innate immune response triggered by *C. jejuni* LPS is a host susceptibility factor in patients with GBS and associated with GBS severity [14]. Although MMN is not associated with preceding *C. jejuni* infection, the multiple phenotypic similarities between the acute motor axonal neuropathy (AMAN) variant of GBS and MMN, i.e. a pure motor neuropathy associated with the presence of conduction block, anti-GM1 antibodies and a response to IVIg but not corticosteroids, suggested the possibility of specific innate immune responses as a susceptibility factor for MMN [30–33]. The lack thereof, combined with the absence of inflammatory markers in blood and cerebrospinal fluid in patients with MMN may suggest that local rather than systemic inflammation is a feature of MMN, in contrast with findings in patients with GBS [12,13,34].

Despite the overall lack of association between innate immune responses and MMN, we observed some interesting patterns for specific proteins. Although the results of the comparison between treatment-naïve patients with MMN and controls should be interpreted with care given the small group of treatment-naïve patients ($n = 7$), we found higher IL-8 baseline concentrations in patients with MMN, independent of IVIg treatment. IL-8 is a pro-inflammatory, neutrophil chemotactic factor produced by a variety of cells after stimulation with other cytokines, such as IL-1β and TNF-α [35]. When comparing patients with MMN and controls, drawing definite conclusions is complicated because of the complex interplay of immunoregulating proteins, in which baseline IL-8 levels could serve as a proxy for other factors, and the limited number of treatment-naïve patients in our study (n = 7). Moreover, within four hours of stimulation with LPS, we found that patients with anti-GM1 IgM antibodies showed an IL-21 increase more often than to patients without anti-GM1 IgM antibodies. The fact that IL-21, a B-cell stimulating and regulating factor, is produced by T cells could explain why not all samples showed a clear IL-21 increase after four hours of stimulation [36,37]. Indeed, other B-cell stimulating or regulating proteins, such as APRIL and BAFF, have previously been shown to emerge only after longer periods of stimulation [15]. Therefore, whether this finding reflects a difference in the timing of the onset of IL-21 increase or preludes a difference in maximum IL-21 production after longer periods of stimulation remains to be uncovered.

To the best of our knowledge, we have performed the first study on innate immune responses to endotoxin of peripheral blood cells in MMN. Considering the rarity of MMN, our patient cohort should be considered large; this allowed us to correlate data on a variety of immunoregulating proteins to detailed clinical parameters, measured at the time of sampling. We believe that our results, obtained after four hours of stimulation with LPS, essentially rule out major general differences in the innate immune response between patients with MMN and controls. However, the results of our study suggest that further research, focusing on products of the innate immune response emerging after longer periods of stimulation with endotoxin, such als IL-21, APRIL of BAFF, may be necessary. The correlation between IVIg dosage at the time of sampling and baseline levels of specific immunomodulating proteins indicates biomarker potential for efficacy of IVIg treatment, but this would need to be addressed further in future studies.

In summary, we demonstrate that altered innate immune responses to LPS are not a susceptibility factor for MMN. As innate immune responses were not associated with the presence of anti-GM1 IgM

antibodies, the results of our study indicate that differences in the innate immune response to endotoxin are unlikely to form part of the immunopathology underlying anti-ganglioside antibody production in MMN.

Author disclosures

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