ABSTRACT: Introduction: Polyneuropathy with immunoglobulin M monoclonal gammapathy (IgM-PNP) is associated with the presence of IgM antibodies against nerve constituents such as myelin associated glycoprotein (MAG) and gangliosides. Methods: To test whether B-cell-stimulating cytokines are increased in IgM-PNP, we measured serum concentrations of 11 cytokines in 81 patients with IgM-PNP and 113 controls. Results: Median interleukin (IL)-6 concentrations were higher in patients with IgM-PNP, and median IL-10 concentrations were higher in the subgroup with anti-MAG IgM antibodies. These serum concentrations were not increased in 110 patients with multifocal motor neuropathy. Discussion: Median IL-6 and IL-10 serum concentrations differ between patients with anti-MAG neuropathy and other patients with IgM-PNP compared with healthy and neuropathy controls. These differences may indicate differences in immune-mediated disease mechanisms. 

Muscle Nerve 000: 000–000, 2019

Cytokines in IgM-PNP

SERUM CYTOKINE PATTERNS IN IMMUNOGLOBULIN M MONOCLONAL GAMMAPOPATHY-ASSOCIATED POLYNEUROPATHY

ABRAHAM C. J. STORK, MD, PhD,1 GER T. RIJKERS, PhD,2 LOTTE VLAM, MD, PhD,3 ELISABETH A. CATS, MD, PhD,4 BEN A. W. DE JONG,5 RUTH D. E. FRITSCH-STORK, MD, PhD,6 JAN H. VELDINK, MD, PhD,7 LEONARD H. VAN DEN BERG, MD, PhD,7 NICOLETTE C. NOTERMANS, MD, PhD,7 and W-LUDO VAN DER POL, MD, PhD7

1 Neurological Department, Hietzing General Hospital with Neurological Center Rosenhügel, Riedelgasse 5, 1130, Vienna, Austria
2 University College Roosevelt, Middelburg, The Netherlands
3 Department of Neurology, Erasmus Medical Center, Rotterdam, The Netherlands
4 Department of Neurology, Gele Hospital, Apeldoorn, The Netherlands
5 Laboratory for Medical Microbiology and Immunology, St Antonius Hospital, Nieuwegein, The Netherlands
6 First Medical Department, Hanusch Hospital, Vienna, Austria
7 Department of Neurology and Neurosurgery, Brain Center Rudolf Magnus, University Medical Center, Utrecht, The Netherlands

Accepted 5 March 2019

ABSTRACT: Introduction: Polyneuropathy with immunoglobulin M monoclonal gammapathy (IgM-PNP) is associated with the presence of IgM antibodies against nerve constituents such as myelin associated glycoprotein (MAG) and gangliosides. The pathogenesis of IgM-PNP is probably dominated by B cells or plasma cells because evidence for T-cell involvement is lacking. The mechanisms underlying B-cell activation and pathogenic antibody production that ultimately cause nerve damage are largely unknown. Immunoglobulin M monoclonal gammapathy-associated polyneuropathy does not respond to treatments that have been successfully used for other demyelinating neuropathies, including intravenous immunoglobulin (IVIg) and cyclophosphamide given with prednisone. Cytokine expression patterns may suggest specific pathophysiological pathways that could help to design novel treatment strategies. We therefore compared expression profiles of cytokines associated with B-cell regulation in patients with IgM-PNP and healthy controls and patients with multifocal motor neuropathy (MMN), another antibody-mediated inflammatory neuropathy, as disease controls.

METHODS

Patients. This study was approved by the medical-ethical committee of the University Medical Center Utrecht. All participants gave written informed consent prior to their inclusion in this study. We collected serum samples of 81 patients with IgM-PNP from a previously reported prospectively followed cohort. Inclusion criteria were polyneuropathy (confirmed in nerve conduction studies) and IgM monoclonal gammapathy, identified by serum electrophoresis with subsequent immunofixation. Excluded were patients with another cause for the neuropathy and patients whose hemato-oncological consultation led to the diagnosis of a hematomal malignancy. All patients underwent an interview and physical examination according to a predefined protocol. Disease severity was expressed by clinical scores, and weakness was measured with a Medical Research Council muscle strength score for 7 proximal and distal muscles in both arms and legs, with a maximum of 140 points for full strength in all muscles. Sensory deficits were quantitated with the inflammatory neuropathy cause and treatment (INCAT) sensory sum score testing pinprick and vibration sensations. Nerve conduction studies were performed according to a previously published protocol, and demyelination was defined according to the American Academy of Neurology ad hoc subcommittee guidelines for chronic inflammatory demyelinating polyradiculoneuropathy (CIDP). Functional deficits were assessed with the INCAT overall disability severity scale (ODSS) functional score. To quantitate disease severity in patients with IgM-PNP and to control for disease duration, functional and clinical scores were assessed 3 years after disease onset. To assess the speed of progression at the moment of cytokine concentration measurement, we compared the clinical and functional scores between the visit at which blood for these measurements was taken and a follow-up assessment 2 years later by the same physician (ACJS). We defined responsiveness to rituximab as improvement in the ODSS functional score 1 year after treatment.

Sera from 113 age- and sex-matched healthy volunteers and from 110 patients with probable or definite MMN according

Abbreviations: APRIL, a proliferation-inducing ligand; BAFF, B-cell-activating factor; CIDP, chronic inflammatory demyelinating polyradiculoneuropathy; ELISA, enzyme-linked immunoassay; IgM, immunoglobulin M; IgM-PNP, immunoglobulin M monoclonal gammapathy-associated polyneuropathy; IL, interleukin; INCAT, inflammatory neuropathy cause and treatment; IVIg, intravenous immunoglobulin; MAG, myelin associated glycoprotein; MGUS, monoclonal gammapathy of unknown significance; MMN, multifocal motor neuropathy (DDSS, overall disability sum score; PNP, polyneuropathy; Th, T helper. Key words: anti-MAG neuropathy; cytokines; monoclonal gammapathy; multifocal motor neuropathy; peripheral neuropathy

Conflicts of Interest: None of the authors have any conflict of interest to disclose

Correspondence to: A. C. J. Stork; e-mail: abraham.stork@wienkav.at

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Published online 00 Month 2019 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/mus.26462
to previously published diagnostic criteria were included as control samples. Patients with MMN were screened for a concomitant monoclonal gammapathy; healthy controls were not screened for paraproteinemia. Seventy-six (69%) patients with MMN were being treated with IVIg maintenance therapy when the serum samples were collected. Sample collection was just before the next IVIg infusion, 2–4 weeks after the last infusion. Serum were frozen immediately and stored at −80°C until analysis.

The presence of anti-MAG antibodies was determined by enzyme-linked immunosorbent assay (ELISA; Bühlmann Laboratories, Schönenbuch, Switzerland). Antiganglioside antibodies were determined as described previously. IgM, immunoglobulin M; IgM-PNP, immunoglobulin M monoclonal gammopathy-associated polyneuropathy; IL, interleukin; INCAT, inflammatory neuropathy cause and treatment; MMN, multifocal motor neuropathy; MRC, Medical Research Council, N/D, not determined; ODSS, overall disability sum score.

RESULTS

Patient Characteristics. Patient characteristics are presented in Table 1. Among the 81 patients with IgM-PNP, 44 had anti-MAG antibodies, and 14 had antibodies against asialo-GM1 or the gangliosides G1, GD1a, GD1b, GM2, or GQ1b. Two patients had antibodies against both MAG and 1 or more gangliosides or asialo-GM1. Twenty-eight patients with IgM-PNP, of whom 19 had anti-MAG antibodies, were treated with rituximab (4 weekly infusions of 375 mg per m² body surface). Two patients with IgM-PNP developed a malignancy within a year after inclusion.

Increased Median IL-6 Concentration in IgM-PNP and Increased Median IL-10 Concentration in Patients With anti-MAG PNP. Direct comparison of concentrations of individual cytokines among patients with IgM-PNP, MMN (IVIg-naive), and controls showed increased (P = 1.3×10⁻⁷) IL-6 concentrations for patients with IgM-PNP. Interleukin-10 concentrations were increased only in patients with IgM-PNP with anti-MAG antibodies (P = 5×10⁻⁵; Table 2, Fig. 1). Cluster analysis did not reveal specific cytokine profiles in patients with IgM-PNP. Compared to healthy controls, IL-6 and IL-10 concentrations were not increased in serum samples from patients with MMN with (n = 6) or without (n = 104) a concomitant monoclonal gammapathy. Reanalyzing our data by comparing cytokine concentrations of only patients with IgM-PNP with demyelinating neuropathy with cytokine concentrations in MMN patients or controls did not yield significantly different results. Notwithstanding the statistically significant differences in median values, IL-6 and IL-10 concentrations were within normal range for most patients with both IgM-PNP and anti-MAG neuropathy.

No Correlation Between Cytokine Concentration and Clinical Characteristics of IgM-PNP. IL-6, IL-10, or other cytokine levels did not differ between patients with progressive or relatively indolent IgM-PNP disease

### Table 1. Patient characteristics. *

<table>
<thead>
<tr>
<th>Patient characteristic</th>
<th>IgM-PNP anti-MAG</th>
<th>IgM-PNP non-MAG</th>
<th>MMN</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at investigation, y</td>
<td>71 (41–88)</td>
<td>62 (45–87)</td>
<td>55 (27–80)</td>
<td>61 (29–95)</td>
</tr>
<tr>
<td>Men</td>
<td>32 (73)</td>
<td>25 (68)</td>
<td>62 (75)</td>
<td>80 (71)</td>
</tr>
<tr>
<td>Demyelinating neuropathy (IgM-PNP) or conduction block (MMN)</td>
<td>28 [68]</td>
<td>26 [81]</td>
<td>110 [100]</td>
<td>0 [0]</td>
</tr>
<tr>
<td>IgM gammopathy</td>
<td>44 [100]</td>
<td>37 [100]</td>
<td>6 [6]</td>
<td>N/D</td>
</tr>
<tr>
<td>INCAT sensory sum score</td>
<td>8 (2–17)</td>
<td>10 (2–18)</td>
<td>138 (125–140)</td>
<td>138 (125–140)</td>
</tr>
<tr>
<td>MRC motor sum score</td>
<td>136 (111–140)</td>
<td>138 (125–140)</td>
<td>3 (1–7)</td>
<td>3 (1–6)</td>
</tr>
</tbody>
</table>

IgM, immunoglobulin M; IgM-PNP, immunoglobulin M monoclonal gammapathy-associated polyneuropathy; IL, interleukin; INCAT, inflammatory neuropathy cause and treatment; MMN, multifocal motor neuropathy; MRC, Medical Research Council, N/D, not determined; ODSS, overall disability sum score.

* Data are median (range) or n [%].
course. Patients in the upper quartile of IL-6 or IL-10 serum concentrations did not differ from other IgM-PNP or anti-MAG neuropathy patients in disease severity, speed of clinical worsening, or presence of demyelinating polyneuropathy in nerve conduction studies. No correlation was found between BAFF, IL-6, or IL-10 concentrations and responsiveness to rituximab treatment.

**Table 2.** Cytokine concentrations for patients with polyneuropathy associated with IgM monoclonal gammopathy, IVIg-naive multifocal motor neuropathy, and controls.†

<table>
<thead>
<tr>
<th>Patient group</th>
<th>IL2, pg/ml</th>
<th>IL4, pg/ml</th>
<th>IL6, pg/ml</th>
<th>IL8, pg/ml</th>
<th>IL10, pg/ml</th>
<th>IL12, pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard range</td>
<td>0.13–2118</td>
<td>0.03–529</td>
<td>0.19–3064</td>
<td>0.16–2545</td>
<td>0.18–2933</td>
<td>0.2–3219</td>
</tr>
<tr>
<td>Anti-MAG PNP</td>
<td>BDL (–20.55)</td>
<td>BDL (–2.81)</td>
<td>1.22 (BDL–33.91)*</td>
<td>1.76 (0.1–16.56)</td>
<td>1.20 (BDL–73.09)*</td>
<td>BDL (–10.95)</td>
</tr>
<tr>
<td>All IgM-PNP</td>
<td>BDL (–20.55)</td>
<td>BDL (–2.81)</td>
<td>0.77 (BDL–33.91)*</td>
<td>1.64 (0.1–44.22)</td>
<td>BDL (–73.09)</td>
<td>BDL (–10.95)</td>
</tr>
<tr>
<td>MMN</td>
<td>BDL (–4.28)</td>
<td>BDL (–0.02)</td>
<td>0.01 (–9.32)</td>
<td>2.38 (BDL–44.89)</td>
<td>0.11 (–104.69)</td>
<td>BDL (–7.1)</td>
</tr>
<tr>
<td>Controls</td>
<td>BDL (–21.41)</td>
<td>BDL (–4.31)</td>
<td>BDL (–5.23)</td>
<td>1.20 (BDL–31.42)</td>
<td>BDL (–12.49)</td>
<td>BDL (–7.49)</td>
</tr>
</tbody>
</table>

†Data are median (range).

**APRIL,** a proliferation-inducing ligand; **BAFF,** B-cell activating factor; **BDL,** below detection limit; **GM-CSF,** granulocyte monocyte-colony stimulating factor; **IFN,** interferon; **IgM,** immunoglobulin M; **IgM-PNP,** IgM monoclonal gammopathy-associated polyneuropathy; **IL,** interleukin; **IVIg,** intravenous immunoglobulin; **MAG,** myelin associated glycoprotein; **MMN,** multifocal motor neuropathy; **PNP,** polyneuropathy; **TNF,** tumor necrosis factor.

*Significantly different from those of healthy controls.

**FIGURE 1.** Interleukin (IL)-6 and IL-10 serum concentrations. IL-6 and IL-10 serum concentrations in pg/ml for different patient groups. Gray bars denote median concentrations. *Significantly different median value. IgM, immunoglobulin M; IVIg, intravenous immunoglobulin; MAG, myelin associated glycoprotein; MAG-PNP, polyneuropathy associated with IgM monoclonal gammopathy with anti-MAG antibodies; MMN, IVIg-naive multifocal motor neuropathy; non-MAG IgM, polyneuropathy associated with IgM monoclonal gammopathy but without anti-MAG antibodies.
DISCUSSION

In this study, patients with IgM-PNP had higher median IL-6 concentrations in serum compared with healthy controls and patients with MMN, whereas patients with IgM-PNP and anti-MAG antibodies (anti-MAG neuropathy) also had elevated median IL-10 concentrations. Immunoglobulin M monoclonal gammopathy-associated polyneuropathy is heterogeneous both in clinical characteristics and in response to treatment, and IL-6 and IL-10 serum concentrations might help in identifying subsets of patients sharing pathophysiological pathways or susceptibility to treatment.

Interleukin-6 is a pleiotropic cytokine with proinflammatory and anti-inflammatory properties. Elevated serum concentrations of IL-6 have been previously reported in patients with other B-cell-associated diseases such as multiple myeloma, Castleman or Schnitzler syndrome, and a minority of patients with monoclonal gammopathy of unknown significance (MGUS) without polyneuropathy. Furthermore, disease activity in B-cell-driven autoimmune diseases such as rheumatoid arthritis or systemic lupus erythematosus as well as in B-cell hematological malignancies has been shown to be correlated with IL-6 serum levels. In our study, patients with IgM-PNP and elevated IL-6 did not have a distinct clinical (e.g., progressive) phenotype. Because follow-up in this study was relatively short, we cannot exclude the possibility that an increased IL-6 concentration may reflect an increased risk of eventually developing a hematological malignancy or a more progressive disease course.

The roles of IL-6 as a survival factor for plasma cell precursors and in skewing T-cell populations toward the proinflammatory T helper (Th) 17 subtype have been postulated as explaining the correlation of elevated IL-6 concentrations and the above mentioned diseases and could be relevant in the pathogenesis of IgM-PNP. Antigenic B-cell stimulation over Toll-like receptors leading to increased IL-6 production and establishment of an IL-6 autocrine loop could be a first step in the monoclonal expansion in plasma cell dyscrasia in IgM-PNP. Elevated IL-6 concentrations could thus be a biomarker for plasma cell dyscrasia rather than for IgM-PNP per se, although the 6 patients with MMN and MGUS in this study did not have elevated IL-6 serum concentrations. A role of IL-6 in IgM-PNP may alternatively lie in skewing T cells toward the proinflammatory Th17 subtype as opposed to T regulatory cells, although this is unlikely because of the lack of evidence of T-cell involvement in this disease.

Patients with anti-MAG neuropathy also had increased serum IL-10 concentrations. These findings are in line with the reported increased IL-10 production by mitogen-stimulated peripheral blood mononuclear cells in 14 patients with anti-MAG neuropathy. Similarly to IL-6, elevated serum IL-10 concentrations have also been described in patients with B-cell dyscrasias without neuropathy. Interleukin-10 not only plays a broadly inhibitory role in the production of Th1 cell and macrophage-produced proinflammatory cytokines but can also activate B cells and promote autoantibody production and is implicated in a growing number of autoimmune diseases.

Increased IL-10 production has been described as a general damage-limiting reaction in neuropathies, but this does not explain why IL-10 was elevated only in patients with anti-MAG neuropathy. The differences in IL-10 concentrations are therefore more likely to reflect differences in pathogenesis between patients with IgM-PNP with or without anti-MAG antibodies.

In contrast to other B-cell mediated or IVIg-responsive neurological diseases, including chronic inflammatory demyelinating polyradiculoneuropathy (CIDP), serum concentrations of the specific B-cell cytokines APRIL and BAFF were not elevated in MMN or patients with IgM-PNP compared with healthy controls. This is in agreement with a previously published report of an IgM-PNP cohort. We did not find the correlation between BAFF serum levels and rituximab responsiveness described by Benedetti et al. The roles that IL-6 and IL-10 play in the pathogenesis of subsets of patients with IgM-PNP deserve additional attention as biomarkers and potential targets for new treatment strategies. Although our data do not support direct use of IL-6 or IL-10 serum concentrations as selection criteria for treatment choices in IgM-PNP or anti-MAG neuropathy, IL-10 concentrations could be included in future studies that seek to predict rituximab responsiveness in anti-MAG neuropathy in addition to other biomarkers such as high BAFF concentrations, lower anti-MAG titers, or FcγRIIIA V/V158 genotype, which have been correlated with a positive response to rituximab. Our data also provide evidence that other biologicals, for example the anti-IL-6R antibody tocilizumab, may be candidates as future treatment options in IgM-PNP.

Ethical Publication Statement: We confirm that we have read the Journal’s position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

REFERENCES


