ANTI-GANGLIOSIDE ANTIBODIES IN PATIENTS WITH RHEUMATOID ARTHRITIS COMPLICATED BY PERIPHERAL NEUROPATHY

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SUMMARY

Gangliosides are a diverse class of glycolipids found in the plasma membrane of mammalian cells and are particularly abundant in cells of the nervous system. Serum antibodies to gangliosides have been detected in various neurological disorders with some evidence that they play a pathogenic role. In this study, we have investigated whether anti-ganglioside antibodies were elevated in a group of patients with rheumatoid arthritis (RA) who developed peripheral neuropathy (PN). An ELISA technique was used to test sera from 28 patients with RA and PN, 38 RA patients without PN and 20 normal controls for the presence of IgG and IgM anti-GM1 and sulphatide antibodies. The patients with RA and PN had higher pain scores ($P < 0.005$), more extra-articular features ($P < 0.05$), higher erosive scores ($P < 0.0001$), lower haemoglobin ($P < 0.005$), higher ESR ($P < 0.001$) and were more often on disease-modifying drugs ($P < 0.05$). Twelve RA patients with PN (43%), but only two RA controls (5%), had positive titres against one or more gangliosides ($P < 0.001$). The neurologic disability score (NDS) correlated with RA duration ($P < 0.05$), and with levels of IgM anti-GM1 ($P < 0.001$) and IgM anti-sulphatide ($P < 0.05$) antibodies. We conclude that PN is more common in patients with severe rheumatoid disease, and a significant proportion have elevated levels of anti-ganglioside antibodies.

KEY WORDS: Rheumatoid arthritis, Peripheral neuropathy, Anti-ganglioside antibodies, Neurologic disability score.

Gangliosides are a family of acidic glycolipids [1] which are composed of lipid (ceramide) and carbohydrate (oligosaccharide chain) moieties. Four gangliosides, GM1, GD1a, GD1b and GT1b, are especially abundant in the brain, while LM1 constitutes the major ganglioside in peripheral nerves [2]. Sulphatide is the major acidic glycosphingolipid in myelin, and in peripheral nerves it is found in concentrations 100 times that of other gangliosides [3]. Gangliosides reside in the outer layer of the plasma membrane where they may regulate diverse physiological processes [4, 5], including neural cell function [6], cell-cell recognition, cell adhesion and the activity of enzymes such as protein kinase C and Na-K-ATPase. They influence neurite outgrowth [7] and possess neuroprotective functions. Previous studies have demonstrated beneficial effects from ganglioside administration in animal models of diabetes, leading to recovery in nerve conduction velocity and maintenance of axonal transport of cytoskeletal proteins [8]. In a study of patients with diabetic peripheral neuropathy (PN), ganglioside administration improved paraesthesiae and nerve conduction [9].

The abundance of gangliosides in the nervous system and their extracellular location make them potential antigenic targets in autoimmune neurological disorders. Antibodies to gangliosides have been found in a variety of neurological conditions, including multifocal motor neuropathy, distal and proximal lower motor neuron syndromes, and occasionally in Guillain-Barré syndrome and polymyositis [10]. The pathogenicity of anti-ganglioside antibodies has been suggested by the development of neuropathy and motor conduction block when these antibodies were injected in rabbits [11]. It has also been found that the IgM anti-GM1 antibodies react against the neuronal membranes by binding to the GM1 ganglioside [12].

Peripheral nerve involvement in rheumatoid arthritis (RA) can include compressive neuropathy, which is by far the commonest, and vasculopathy, resulting in distal sensory and combined sensorimotor neuropathy in 1–18% of patients [13]. Although the underlying pathology of rheumatoid neuropathy is not clear, humoral mechanisms such as the deposition of immune complexes and fixation of complement are thought to be important factors.

Histological examination of sural nerves has demonstrated deposition of IgG, IgM, complement and fibrin in areas corresponding to those of fibrinoid necrosis [14]. As far as we are aware, no previous studies have examined the levels of anti-ganglioside antibodies in patients with RA and peripheral neuropathy.

In this study, we have investigated the prevalence of anti-GM1 and sulphatide antibodies in patients with RA complicated by PN, and compared the results with measures of rheumatoid disease activity and damage.

PATIENTS AND METHODS

Consecutive patients with RA defined according to the ARA 1987 revised criteria [15], who were attending a district general hospital out-patient rheumatology
TABLE I
Demographic details and indices of disease activity and damage in RA patients with and without peripheral neuropathy. Values given with confidence limits are means ± S.D.

<table>
<thead>
<tr>
<th></th>
<th>PN (n = 28)</th>
<th>RA controls (n = 38)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male:female</td>
<td>10:18</td>
<td>8:30</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>64 ± 8</td>
<td>60 ± 9</td>
</tr>
<tr>
<td>Clinical indices</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of RA (yr)</td>
<td>11.4 ± 5.7</td>
<td>9 ± 6.2</td>
</tr>
<tr>
<td>Extra-articular</td>
<td>n = 6</td>
<td>n = 4</td>
</tr>
<tr>
<td>vasculitis</td>
<td>n = 7</td>
<td>n = 3</td>
</tr>
<tr>
<td>Early morning stiffness (min)</td>
<td>45 ± 32</td>
<td>34 ± 29</td>
</tr>
<tr>
<td>Visual analogue score (mm)</td>
<td>65 ± 17***</td>
<td>49 ± 23</td>
</tr>
<tr>
<td>Disease-modifying therapy</td>
<td>n = 21*</td>
<td>n = 17</td>
</tr>
<tr>
<td>Lower limb operations</td>
<td>n = 13</td>
<td>n = 10</td>
</tr>
<tr>
<td>Laboratory indices</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>11.9 ± 1.4***</td>
<td>13.3 ± 1.8</td>
</tr>
<tr>
<td>ESR (mm/h)</td>
<td>53 ± 28****</td>
<td>28 ± 25</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>41 ± 38</td>
<td>35 ± 64</td>
</tr>
<tr>
<td>Rheumatoid factor</td>
<td>228 ± 276</td>
<td>166 ± 274</td>
</tr>
<tr>
<td>ANA</td>
<td>345 ± 809</td>
<td>261 ± 730</td>
</tr>
<tr>
<td>IgG (gm/l)</td>
<td>106 ± 50</td>
<td>142 ± 33</td>
</tr>
<tr>
<td>IgM (gm/l)</td>
<td>171 ± 75</td>
<td>157 ± 125</td>
</tr>
<tr>
<td>Larsen radiological score</td>
<td>80 ± 28****</td>
<td>44 ± 30</td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.01, ***P < 0.005, ****P < 0.001, *****P < 0.0005.

The following measures of RA disease activity and damage were recorded: duration of early morning stiffness, Ritchie articular index, 10 cm visual analogue scale for pain, presence of lower limb operations and extra-articular manifestations, and past or present medications with disease-modifying therapy.

Of the 28 patients with PN, 13 had extra-articular manifestations of RA, and of these seven patients had cutaneous, nail fold vasculitis or vasculitic ulcers on biopsy, while the remaining six had rheumatoid pulmonary complications, such as fibrosis, nodules and pleural effusion. One patient had Felty's syndrome.

Blood was taken for estimation of haemoglobin, ESR, CRP, IgM RF by ELISA, ANA, ANCA, cryoglobulins, immunoglobulins, complement, vitamin B12, folate, creatinine, thyroid-stimulating hormone, hepatic enzymes, blood glucose and anti-ganglioside antibodies. A standard chest radiograph was performed, and hands and feet films were graded by the Larsen score.

Patients with compression neuropathy or who had an alternative cause for PN, e.g. metabolic, infective, toxic or hereditary, were excluded. Four RA patients with diabetes and alcohol abuse were excluded.

![Fig. 1.—Serum levels of IgG anti-sulphatide antibodies in patients with rheumatoid arthritis and peripheral neuropathy (PN), rheumatoid arthritis without peripheral neuropathy (RA) and normals (values shown are in arbitrary units). Values > 2 s.d. of the mean for the RA controls were considered abnormal. —— mean for the RA controls + 2 s.d.; — mean for the PN, RA, normals.](image-url)
Enzyme-linked immunosorbent assay (ELISA)

A previously described technique was modified [17]. Commercially available (Sigma) bovine gangliosides GM1 and sulphatide were added to plastic microtitre plates at a concentration of 1 µg/ml in ethanol. After allowing ethanol to evaporate overnight at 4°C, the plate was washed with phosphate-buffered saline (PBS)/0.05% Tween and blocked by 0.1% human serum albumin in PBS/Tween for 1 h. Each patient serum diluted 1:100 in PBS/Tween was added to duplicate wells and incubated for 2 h at room temperature. PBS/Tween alone was added to blank wells. A high and low control serum sample was incubated in each plate. After washing, alkaline phosphatase-conjugated anti-human IgG or IgM (1:1000 in PBS/Tween + 1% goat serum) was...
FIG. 4.—Serum levels of IgM anti-GM1 antibodies in patients with rheumatoid arthritis and peripheral neuropathy (PN), rheumatoid arthritis without peripheral neuropathy (RA) and normals (values shown are in arbitrary units). Values > 2 S.D. of the mean for the RA controls were considered abnormal. —— mean for the RA controls + 2 S.D.; — mean for the PN, RA, normals.

Mann–Whitney U-test and correlations by Spearman’s rank correlation.

RESULTS

Twenty-eight patients with PN were compared with the RA control group (Table I). The neuropathic group had a higher pain score ($P < 0.005$), more extra-articular features ($P < 0.05$), higher erosive scores ($P < 0.0001$), lower haemoglobin ($P < 0.005$), higher ESR ($P < 0.001$) and were more frequently on disease-modifying drugs ($P < 0.05$). However, age, RA disease duration, lower limb operations, early morning stiffness, CRP, rheumatoid factor, ANA and complement levels were not significantly different between the neuropathic group and their RA controls. RA patients with PN had significantly higher IgG anti-sulphatide antibody levels than RA controls ($P < 0.005$) and HV ($P < 0.0001$). Levels of IgM anti-sulphatide antibodies were also raised among RA neuropathy patients compared to RA controls ($P < 0.05$) and HV ($P < 0.001$). IgG anti-GM1 antibody levels were significantly higher in the neuropathic group than in the HV ($P < 0.0001$), but were not significantly greater than those in RA controls ($P = 0.09$). There were no statistical differences between the three groups of patients for IgM anti-GM1 antibody levels, although the neuropathic group had a higher mean value.

Twelve patients (43%) with RA and PN compared to two patients (5%) from the RA control group had abnormal antibody levels against one or more gangliosides ($P < 0.001$), but none of the healthy volunteers had elevated levels, according to criteria used in this study (Figs 1–4). Seven patients with RA

added at 100 μl/well for 1 h at room temperature. The plate was washed and incubated with 100 μl/well of 1 mg/ml p-nitrophenyl phosphate in diethanolamine buffer (Sigma) for 2 h (GM1) or 30 min (sulphatide) at room temperature.

A total of 50 μl of 3 M NaOH was added to each well to stop the reaction. The absorbance or optical density (OD) was read at 405 nm by an ELISA plate reader (Titertek Multiskan Plus MK.11). A reference serum with high levels of antibodies was used to obtain a standard curve from which arbitrary units were determined. Values greater than two standard deviations of the mean for the RA control patients were considered to be abnormal. The intra- and interassay coefficients of variation were between 7.1 and 9.5% and 8.9 and 13.9%, respectively, for the four assays.

Absorption of sera with sulphatide

To test for any cross-reactivity between antibodies to sulphatide and GM1, diluted sera (1:100) from 20 RA patients with PN were incubated overnight with or without 100 μg/ml sulphatide in PBS/Tween.

An ELISA was then performed, as described above, to determine levels of IgM GM1 antibodies with and without sulphatide absorption.

The percentage of antibody activity remaining after absorption was calculated as follows:

$$\frac{\text{OD (serum + sulphatide)} - \text{OD (blank)}}{\text{OD (serum only)} - \text{OD (blank)}} \times 100$$

Statistical analysis

The significance of differences between the neuropathy and control groups was tested by the
and PN had abnormal antibody levels against more than one ganglioside.

There were significant correlations between IgM anti-GM1 and IgM anti-sulphatide antibodies ($P < 0.0005$), and between IgM anti-GM1 and IgG anti-sulphatide antibodies ($P < 0.0001$). Four of 20 sera (20%) from RA patients with PN absorbed with sulphatide demonstrated a $\geq 50\%$ reduction in IgM anti-GM1 antibody levels. However, the majority of sera (65%) showed little change ($<5\%$) in IgM GM1 binding.

Those patients who demonstrated high anti-ganglioside antibody levels had lower C4 complement levels ($P < 0.05$). There was no significant correlation between the anti-ganglioside antibody levels and rheumatoid factor, and measures of RA disease activity or damage. Four patients with PN and elevated levels of anti-ganglioside antibodies had clinical evidence of vasculitis.

Neurophysiological studies revealed axonal polyneuropathy and mononeuritis multiplex in 10 (36%) patients. Clinical sensorimotor neuropathy was found in 23 patients (82%), while pure motor or sensory neuropathy was observed in two (7%) and three (11%) patients, respectively. The NDS correlated with RA duration ($P < 0.05$), and with the presence of IgM GM1 ($P < 0.005$) (Fig. 5) and IgM sulphatide ($P < 0.05$) antibodies.

**DISCUSSION**

We have demonstrated that a significant proportion of RA patients with PN (43%) had abnormal anti-ganglioside antibody levels. In contrast, only 5% of RA patients without PN had elevated levels, while only low levels were found in sera of healthy volunteers. We found that PN occurs more frequently in patients with severe RA, which is in agreement with other studies [18].

We did not find a relationship between the anti-ganglioside antibodies tested and a clinical subset of neuropathy. This is explained by the majority of patients having a mixed sensorimotor presentation, and only a small number of patients having a pure motor or sensory neuropathy. Similarly, we are not able to relate the presence of the anti-gangliosides to axonal or demyelination changes because of the smaller number of patients with pure axonal features.

The presence of PN in patients with RA can be difficult to recognize as patients often relate neurological symptoms to joint disease. It is also difficult to assess the neurological system in the presence of severe joint disease. In this study, we used the NDS for the purpose of assessing severity and quantitating neurological deficit. Because the NDS is a global score of muscle weakness, reflex and sensory abnormality, it is thought to be one of the more robust measures of global neurological deficit, and its usefulness has been demonstrated in a trial of plasma exchange in patients with chronic inflammatory demyelinating polyneuropathy [19]. The sensitivity and reproducibility of NDS are established [20, 21].

In our study, the presence of rheumatoid factor was not different in patients with and without PN, which suggests that it is not an important factor in the aetiology of neuropathy. There was evidence of complement C4 consumption in those neuropathy patients with high anti-ganglioside antibodies, suggesting that complement activation may play a pathogenic role.

Antibodies may be pathogenic or arise as a result of non-specific damage to neuronal tissues. It is not clear why patients with RA complicated by PN develop...
antibodies against gangliosides. It could relate to failure of tolerance by peripheral T cells or be a result of molecular mimicry, as the ganglioside carbohydrate sequence is shared with bacterial lipopolysaccharide [22]. However, greater neurological deficit, as measured by the NDS, in those patients with higher anti-IgM antibodies against GM1 and sulphatide molecules, indicates that these antibodies in RA PN are partly related to the severity of neuronal tissue breakdown.

Other clinical syndromes have been associated with defined anti-ganglioside specificity. IgM anti-GM1 antibodies are found in 60–80% of patients with multifocal motor neuropathy [23] and IgM anti-sulphatide antibodies have been described in chronic axonal sensory neuropathy [24], while IgG anti-GQ1b antibodies are detected in patients with Miller Fisher syndrome [25]. In the present study, seven of the 12 patients (58%) with anti-ganglioside antibodies had antibodies to both gangliosides, with significant correlations between the IgM and IgG antibodies to both gangliosides. The absorption experiments confirmed IgM cross-reactivity with both gangliosides in only 20% of the sera tested. This suggests that in the majority of patients clones of activated B cells are present which may produce antibodies to different gangliosides. These observations are in agreement with their relationship with the progression of neuronal pathology.

In conclusion, measurement of anti-ganglioside antibodies may prove to be helpful in the detection and assessment of PN in RA. Further work is needed to determine the pathogenicity of these antibodies and their relationship with the progression of neuronal pathology.

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REFERENCES

