Antibodies to neuroblastoma cells in rheumatoid arthritis: 
A potential marker for neuropathy

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Abstract

Objective
To investigate the prevalence of antibodies to neuroblastoma cells in patients with rheumatoid arthritis (RA) complicated by peripheral neuropathy (PN), and to determine whether there is any relationship of these antibodies with the severity of neuropathy.

Methods
The study was carried out on 28 patients with RA complicated by PN, 29 RA patients without PN and 28 healthy volunteers (HV). A cell-based ELISA method was used to test sera for the presence of IgG and IgM anti-neuroblastoma cell antibodies. Localisation and characterisation of neuroblastoma antigens recognised by patients' sera was carried out by immunofluorescent microscopy and Western blotting.

Results
Elevated levels of IgG anti-neuroblastoma cell antibodies were found in 10 (36%) neuropathic patients and in 1 (3%) RA control (χ² = 9.53, P = 0.002), while significant levels of IgM anti-neuroblastoma cell antibodies were demonstrated in 10 (36%) neuropathic patients and in 2 (7%) RA controls (χ² = 7.12, P = 0.008). Overall, the levels of antibodies in healthy volunteers were significantly lower than in RA controls and patients with PN. No significant relationship was found between the level of anti-neuroblastoma cell antibodies and severity of RA or neuropathy. Immunofluorescence staining of neuroblastoma cells with sera from 18 neuropathic patients demonstrated cytoplasmic and/or nuclear patterns. Western blotting demonstrated reactivity with a heterogeneous group of neuroblastoma antigens. Little or no reactivity was seen with RA control or HV sera.

Conclusion
Antibodies against neuroblastoma cells are more prevalent in RA patients with peripheral neuropathy than in RA patients without peripheral nerve involvement. Such antibodies may be useful diagnostic markers for peripheral neuropathy in RA.

Key words
Rheumatoid arthritis, peripheral neuropathy, neuroblastoma antibodies.
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Introduction
Rheumatoid arthritis is a chronic systemic inflammatory disorder where genetic and immunologic factors play a role in the pathogenesis and progression of the disease. Increased autoantibody production, elevated levels of immune complexes and evidence of classical pathway complement activation at the site of disease implies that the systemic manifestations of RA are largely mediated by B cell overactivity. Extra-articular features may be evident in 10 - 20% of patients, and neurologic manifestations as a result of peripheral and central nervous system involvement are one of the more common. Peripheral nerve involvement in RA can include compressive neuropathy, which is by far the commonest, and vasculopathy, resulting in distal sensory and combined sensorimotor neuropathy (1, 2). Though the underlying pathology of rheumatoid neuropathy is not clear, humoral mechanisms such as deposition of IgG, IgM, complement and fibrin in areas corresponding to those of fibrinoid necrosis in sural nerve biopsies have been demonstrated (3, 4).

It can be difficult to evaluate peripheral neuropathy clinically in some RA patients and, because of the reproducibility problems associated with neurophysiology examination, identification of a serological marker would be useful. In recent studies we have shown that increased levels of antibodies to antigens found in peripheral nerves are detectable in some RA patients with peripheral neuropathy. These include antibodies to gangliosides (5) found in the nerve plasma membrane, and neurofilament polypeptides which are major structural proteins of the neuronal cytoskeleton (6). Such markers may be useful in the diagnosis of PN in RA, though their sensitivity is low since only 30-40% of patients had elevated antibody levels, and no relationship was found between these particular autoantibodies and the severity of RA or neuropathy.

In our search for other diagnostic and prognostic markers in PN we decided to look for antibodies directed against whole nerve cells. We chose to use a neuroblastoma cell line (SK.N.SH) which has been widely employed in neurological diagnosis and research. Anti-neuroblastoma cell antibodies have been described in various autoimmune neurological disorders and in inflammatory polyneuropathy. Sher et al. have shown the ability of neuroblastoma cell lines to express the voltage-operated calcium channels thought to be recognised by antibodies in the Lambert-Eaton myasthenic syndrome (7). Van Doorn et al. reported anti-neuroblastoma cell antibodies in 42% of patients with inflammatory demyelinating polyneuropathy using an immunofluorescence technique (8). Others suggested that spontaneous or intravenous human immunoglobulin-induced recovery in inflammatory demyelinating polyneuropathy is associated with the increased production of anti-idiotypic antibodies against anti-neuroblastoma cell antibodies (9, 10). In these studies the antigenic determinants of these antibodies were not elucidated, though some have suggested that anti-neuroblastoma cell antibodies may be directed toward carbohydrate determinants expressed on neutral glycolipids. The main aims of this study were to examine whether antibodies directed against neuroblastoma cell antigens are present in patients with RA complicated by peripheral neuropathy, and to investigate whether the levels of these antibodies show any relationship with the severity of neuropathy.

Materials and methods
Patients
Consecutive patients with RA defined according to the ARA (American Rheumatism Association, now the American College of Rheumatology) 1987 revised criteria (11) were assessed for the presence of peripheral neuropathy. Some of these patients have been described previously (5, 6). The diagnosis was supported by nerve conduction studies, which included recording of the motor latency, amplitude, conduction velocity and F wave of the median, ulnar and peroneal nerves bilaterally. The sensory latency and amplitude of the median, ulnar and sural nerves bilaterally were also recorded. In addition, an electromyogram (EMG) of the tibialis anterior was performed.

The patients’ neurological symptoms and signs were assessed using the neuropathic symptom score (N.S.S.) and the neurologic disability score (N.D.S.) of
Dyck et al. (12). The N.S.S. is derived from a neurologic history obtained using a standardized procedure. Selected symptoms which occur in neuropathy are scored as present (1) or absent (0), with the total score being a summation of weakness, sensory and autonomic symptoms. The N.D.S. is a measure of neurologic deficit and includes evaluation of the cranial nerves, strength, deep tendon reflexes, and sensory subsets. Strength is scored from 0 for normal power to 4 for complete weakness, while reflexes and sensation are scored 0 for normal, 1 for decreased and 2 for absent responses. Of the 28 patients with peripheral neuropathy, 13 had extra-articular manifestations of RA, and of these 7 had cutaneous nailfold vasculitis or vasculitic ulcers on biopsy, while the remaining 6 had rheumatoid pulmonary complications such as fibrosis, nodules and pleural effusion. One patient had Felty’s syndrome. Patients with compression neuropathy or who had an alternative cause for peripheral neuropathy (for example, metabolic, infective, toxic or hereditary) were excluded. Four RA patients with diabetes and alcohol abuse were also excluded. Local ethical committee approval was obtained for this study.

Twenty-nine consecutive RA patients without clinical symptoms or signs of peripheral neuropathy, as judged by the NSS and NDS, and 28 healthy volunteers were recruited as controls. None of the patients declined to participate. The following clinical and laboratory measures of RA disease activity and damage were obtained; extra-articular manifestations, past or present medications with disease-modifying therapy, haemoglobin, ESR, CRP and IgM RF by ELISA. Blood was taken for evaluation of ANA, ANCA, cryoglobulins, immunoglobulins, complement, vitamin B12, folate, creatinine, thyroid stimulating hormone, hepatic enzymes, and blood glucose. Standard chest radiograph was performed and films of the hands and feet were graded by the Larsen score.

**Anti-neuroblastoma cell antibody ELISA**

The human neuroblastoma cell line SK.N.SH was obtained from the European Collection of Cell Cultures, Centre for Applied Microbiology and Research, Salisbury, UK. Cells were maintained in Dulbecco’s Modified Eagle Medium (DMEM) with 10% foetal calf serum, 2 mM glutamine and 1 mM penicillin/streptomycin (complete culture medium, CCM).

We developed the following ELISA technique for detection of anti-neuroblastoma cell antibodies. The neuroblastoma cells were removed from the culture flask using 0.25% Trypsin with 0.1 mM EDTA in calcium- and magnesium-free Earle’s balanced salt solution (EBSS). Cells were centrifuged, washed and suspended in CCM. Each well of a 96-well plate was seeded with 1 x 10^5 cells in 200 µl CCM and the plate was incubated overnight at 37°C. The CCM was removed and, after washing the wells with EBSS, was replaced with a serum-free mixture of DMEM and Ham’s F12 nutrient medium (1:1) for 24 hours.

Cells were then fixed by adding 100 µl/well of ice-cold methanol for 10 minutes and washing 3 times manually with PBS. Non-specific binding sites were blocked by adding 100 µl/well of 1% bovine serum albumin for 1 hour at room temperature. Sera diluted at 1:50 in PBS were incubated in duplicate at 100 µl well and incubated for 2 hours at room temperature. PBS alone was added to blank wells. A high and a low control serum sample was also incubated in each plate.

After washing 3 times in PBS, 100 µl/well of anti-human IgG ([Sigma] 1:5000 in PBS + 1% goat serum) or IgM ([Sigma] 1:1000 in PBS + 1% goat serum) conjugated to alkaline phosphatase was added and the plates were incubated for 1 hour at room temperature and then washed 3 times in PBS. 100 µl per well of 1 mg/ml p-nitrophenyl phosphate in diethanolamine buffer (Sigma) was added for 30 minutes at room temperature and the reaction was stopped by adding 50 µl 3M sodium hydroxide (NaOH) to each well.

The absorbance or optical density (OD) was read at 405 nm by an ELISA plate reader (Titertek Multiskan Plus MK11). A reference serum from a PN patient with high levels of antibodies was used to obtain a standard curve from which arbitrary units were determined. The patient chosen gave the maximum values in both the IgG and IgM assays. Values more than 2.5 SD above the mean for the RA controls were considered to be elevated. We compared patients with disease controls to see if there was any difference between RA patients with and without PN. Sera from one PN patient with high levels and one with low levels were used as controls on every plate. The intra- and interassay coefficients of variation were between 6% - 7.5% and 7% - 11.6%, respectively, for all assays. As a control we also carried out ELISAs with the same sera on non-neural cells. For this we used the same method as above on synovial fibroblasts derived from a patient with RA. Cells were obtained and cultured as described previously (13).

**Immunofluorescence staining**

The neuroblastoma cells were trypsinized, washed in complete culture medium and 2 ml of the cell suspension (8 x 10^6/ml) was seeded onto coverslips in a 35 mm Petri dish. The cells were cultured overnight on 13 mm coverslips and were permeabilised by fixing in ice cold methanol for 5 minutes. After washing 3 times in PBS, 20 µl of patients’ sera (diluted 1:50 in PBS) were added to each coverslip and these were incubated at room temperature for 1 hour. In some cases cells were fixed in 3.5% formaldehyde and stained without permeabilisation, or were stained live by incubating with patients’ sera at 4°C. The coverslips were washed in PBS, and then 20 µl of 1:500 fluorescein isothiocyanate (FITC)-conjugated anti-human polyclonal immunoglobulin as a second antibody was added for 1 hour at room temperature. Washing was then repeated in PBS and the specimens were mounted in PBS/glycerol (1:9) and DABCO (114-diazabicyclo-[2.2.2-octane]) to prevent fading of the fluorescence. The slides were read blindly and scored using set criteria by two independent laboratory technicians with considerable experience in immunofluorescence methods. In order to compare the staining of neuroblastoma cells with that of another cell type, we also carried out the above procedure on synovial fibroblasts from one patient with RA and one with OA.

**Western blotting**

Neuroblastoma cells were cultured in CCM in 60 mm tissue culture dishes until...
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cellular (usually 2 days). We found in preliminary experiments that the sera from some patients showed reactivity with bovine serum proteins present in the CCM. Therefore, to avoid contamination with these proteins in the Western blotting, the CCM was decanted and the cells were washed with EBSS and incubated with serum-free DMEM/Ham’s F12 medium containing glutamine and penicillin/streptomycin for a further 24 hours before being solubilised in 1 ml of hot sample buffer. Electrophoresis of the samples was carried out on 12% SDS-polyacrylamide gels run under reducing conditions at 200 volts for 45 minutes. The separated neuroblastoma cell proteins were transferred electrophoretically onto nitrocellulose and blocked overnight in 3% gelatin. After washing 3 times with PBS/0.2% Tween, the nitrocellulose paper was cut into vertical strips and incubated with patient sera diluted 1:100 in PBS/Tween and 1% gelatin at room temperature for 2 hours. They were then washed 3 times in citrate buffered saline (TCBS) and incubated with Protein G conjugated to horseradish peroxidase at a 1: 3.000 dilution with TCBS and 1% gelatin. The strips were then washed 6 times in PBS/Tween. Protein bands were visualised using an enhanced chemiluminescence technique (ECL-Amersham International).

Statistical analysis
The significance of differences between the neuropathy and control groups was tested by one-way analysis of variance with correction for multiple comparisons using the Kruskal-Wallis Z procedure. Correlations were examined by Spearman’s rank method. The frequencies of patients with abnormal antibody levels in the neuropathy and control groups were compared using \( \chi^2 \) tests. All analyses were carried out using the Number Cruncher Statistical Package for Windows (NCSS vs. 6.0.4).

Results
Twenty-eight RA patients with peripheral neuropathy were compared with the RA control group. Neurophysiological studies revealed pure axonal polyneuropathy or mononeuropathy multiplex in 10 (36%) patients. Clinical sensorimotor neuropathy was found in 23 patients (82%), while pure motor or sensory neuropathy was observed in 2 (7%) and 3 (11%) patients, respectively. Demographic and indices of disease activity and damage in RA patients with and without peripheral neuropathy (PN) are shown in Table I. Three patients from the neuropathic group and 5 from the RA control group were taking corticosteroids.

Table I. Demographic details and indices of disease activity and damage in RA patients with and without peripheral neuropathy (PN).

<table>
<thead>
<tr>
<th></th>
<th>RA + PN (n = 28)</th>
<th>RA controls (n = 29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male : Female</td>
<td>10 : 18</td>
<td>7 : 22</td>
</tr>
<tr>
<td>Age (yrs.)</td>
<td>64 (8)</td>
<td>61 (9)</td>
</tr>
<tr>
<td>Clinical indices</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of RA (yrs.)</td>
<td>11.4 (5.7)</td>
<td>10 (6.2)</td>
</tr>
<tr>
<td>Extra-articular</td>
<td>n = 13*</td>
<td>n = 7</td>
</tr>
<tr>
<td>Disease modifying therapy</td>
<td>n = 21*</td>
<td>n = 17</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>29 (25)</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>41 (38)</td>
<td>35 (64)</td>
</tr>
<tr>
<td>Rheumatoid factor (% +ve.)</td>
<td>66%</td>
<td>39%</td>
</tr>
<tr>
<td>ANA (% +ve)</td>
<td>25%</td>
<td>17%</td>
</tr>
<tr>
<td>pANCA (% +ve)</td>
<td>28.5%</td>
<td>10%</td>
</tr>
<tr>
<td>Ig G (gm/l)</td>
<td>166 (50)</td>
<td>142 (33)</td>
</tr>
<tr>
<td>Ig M (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>

Values given are means (SD).
* p < 0.05, ** p < 0.005, *** p < 0.001, **** p < 0.0005.

Anti-neuroblastoma cell antibodies
The RA patients with PN had significantly higher levels of IgG anti-neuroblastoma cell antibodies than both RA patients without PN and healthy volunteers (F = 24.89, P < 0.0001). Compared with RA controls, elevated levels of IgG anti-neuroblastoma cell antibodies were found in 10 (36%) neuropathic patients.
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and 1 (3%) RA control ($\chi^2 = 9.53, P = 0.002$), while raised levels of IgM anti-neuroblastoma cell antibodies were detected in 10 (36%) neuropathic patients and 2 (7%) RA controls ($\chi^2 = 7.12, P = 0.008$) (Figs. 1, 2). Overall, 14 (50%) neuropathic patients and 3 (10%) RA controls had elevated levels of either IgM or IgG anti-neuroblastoma cell antibodies ($\chi^2 = 10.70, P = 0.001$). Six patients had significant levels of both IgG and IgM anti-neuroblastoma cell antibodies. The levels of IgG and IgM antibodies were significantly lower (all $p$ values < 0.001) in the HV compared to both RA controls and patients with PN. Control ELISAs carried out on synovial fibroblasts revealed a range of reactivity with sera from all groups, but there were no overall differences in the level of IgG or IgM antibodies to these cells between the neuropathy and control groups (data not shown). There were no correlations between ELISA reactivity with synovial fibroblasts and neuroblastoma cells, and some non-neuropathic sera had high reactivity in the fibroblast but not the neuroblastoma assay. This may reflect the reactivity of some RA control sera with non-neural antigens (see below) which are not found in neuroblastoma cells.

There was no correlation between the levels of IgG or IgM anti-neuroblastoma cell antibodies and clinical and laboratory measures of RA disease activity or damage, nor was there any correlation between the levels of these antibodies and the titres of ANA, ANCA, IgG or IgM rheumatoid factor, total serum IgG and IgM. We also examined whether there were any differences between patients depending on RF positivity. In PN no significant differences were found between RF+ and RF- patients for IgG anti-neuroblastoma antibodies (4.83 vs. 3.5) or for IgM anti-neuroblastoma antibodies (7.3 vs. 4.9). However, RF+ patients with PN had significantly higher levels of IgG (4.83 vs. 2.38, $p = 0.02$) and IgM (7.3 vs. 2.11, $p = 0.001$) anti-neuroblastoma antibodies than RF+ patients without PN. It was not possible to compare the levels in ANA+ patients because of the low numbers involved. Cross-sectional analysis did not reveal any correlation between the neurologic disability score and the levels of anti-neuroblastoma cell antibodies. However, in a small number of patients ($n = 3$) who underwent sequential examination we did find a close relationship between the severity of neuropathy and antibody levels against neuroblastoma cells, with significant drops in antibody levels occurring as the N.D.S. improved (Fig. 3).

Correlation with other antibodies against neural antigens

We have demonstrated previously that antibodies to gangliosides (5) and neurofilaments (6) are raised in patients with peripheral neuropathy, and 26/28 of the PN patients in this study had such raised antibodies. We therefore examined whether there was any correlation between anti-neuroblastoma antibodies and those against gangliosides and neurofilaments. Weak correlations were found between IgG anti-neuroblastoma antibodies and IgG anti-GM1 ganglioside antibodies ($r = 0.42, p = 0.03$), and anti-sulphatide antibodies ($r = 0.48, p = 0.01$). No correlations were found with anti-neurofilament antibodies. In the case of IgM anti-neuroblastoma antibodies, no correlations were found with IgM anti-ganglioside or anti-neurofilament antibodies. Furthermore, 5 of the 26 patients with abnor-

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**Fig. 2.** Serum levels of IgM anti-neuroblastoma cell antibodies in patients with RA and peripheral neuropathy (PN), in RA patients without peripheral neuropathy (RA), and in healthy volunteers (HV). Values shown are in arbitrary units. Values greater than 2 SD of the mean for RA controls were considered abnormal. (-----) mean for the RA controls + 2 SD; (——) mean for the PN, RA, HV subjects.

**Fig. 3.** X-Y plot. Sequential data on 3 RA patients with peripheral neuropathy showing the relationship between neurologic disability score (NDS) and serum IgG antibody levels against neuroblastoma cells on different occasions. Closed symbols represent NDS values. Open symbols represent IgG anti-neuroblastoma cell antibodies.
mal anti-ganglioside or anti-neurofila-
ment antibodies had anti-neuroblastoma
antibodies within the normal range, sug-
gesting that antibodies against neurob-
lastoma cells may recognise a different
set of antigens and therefore identify a
different group of RA patients with pe-
ripheral neuropathy. Interestingly, by
combining all patients with elevated IgG
or IgM antibodies against at least one
antigen (GM1, neurofilaments or neuro-
blastoma cells) it was possible to iden-
tify 73% of the patients with PN. Only
18% of the RA controls had raised anti-
body levels against one or more of these
antigens.

**Immunofluorescence microscopy**

Immunofluorescence staining was per-
formed with the sera of 18 patients with
PN, 9 RA patients without neuropathy
and 8 healthy volunteers. The intensity of
staining was far greater in the neuro-
pathic group compared with the RA and
healthy control groups (Table II). In the
sera tested there was a strong correla-
tion between the intensity of staining by
immunofluorescence and the ELISA re-
results ($r = 0.78$, $P < 0.0001$). With sera
from the PN patients the pattern of stain-
ing in the neuroblastoma cells was usual-
ly cytoplasmic (Fig. 4a), although speck-
led staining of the nucleus was also seen
with a minority of sera ($n = 3$, Fig. 4b).
Little or no cytoplasmic staining was
seen with the RA control sera, although
nuclear staining was observed in some
cases (Fig. 4c). Staining of neuroblas-
toma cells with sera from healthy vol-
unteers was usually very weak or absent
(Fig. 4d). None of the sera stained the
surface of the cells, either live or after
formaldehyde fixation.

Staining of permeabilised synovial fibro-
blasts gave a variety of staining patterns
including staining of the nucleus and/or
cytoplasmic filaments (not shown).
Staining was seen with sera from both
the PN and RA control groups, although
little or no staining was seen with nor-
mal sera. Such patterns are consistent
with previous reports which have shown
staining of the nucleus and/or vimentin
intermediate filament network with sera
from RA patients. The pattern of stain-
ing of neuroblastoma cells with PN sera
was different to that on synovial fibro-
blasts, apart from 3 sera which showed
nuclear staining in both cell types. The
two slide readers agreed on > 90% of
the results.

**Western blotting**

Western blotting of neuroblastoma anti-
gens with sera from 12 patients with PN
showed a variety of reactive bands, al-
though no particular bands were recog-
nised by all sera. Common bands were
recognised by some sera, particularly at
~ 36 kDa, 42 kDa, 45 kDa and 48 kDa,
although prominent bands were also seen
at ~ 65 kDa and 85 kDa in individual
sera. Typical blots are shown in Figure
5. Reactivity with some bands was seen
in 6 RA sera, but this was generally much
weaker than with the PN sera. Little or
no reactivity was seen with sera from 6
healthy controls.
Discussion

We found significantly higher levels of anti-neuroblastoma cell antibodies among RA patients with peripheral neuropathy than among RA and HV controls. In RA peripheral neuropathy the serum reactivity against neuroblastoma antigens seems to be a specific immune response. This was suggested firstly by the lack of any relationship between the levels of anti-neuroblastoma cell antibodies and total serum IgG or IgM. Secondly, high levels of anti-neuroblastoma antibodies were not merely a reflection of RF positivity since the levels were significantly higher in RF+ patients with PN than in RF+ patients without PN. Thirdly, the absence of a correlation between the anti-neuroblastoma cell antibodies and measures of RA severity indicate that the immunological response to neuroblastoma cells may have a pathogenic relationship to the peripheral neuropathy. Such a relationship was indicated in 3 patients in whom we were able to collect data before treatment was initiated. However, on cross-sectional analysis the anti-neuroblastoma cell antibody levels showed no relationship with the severity of neuropathy and did not correlate with the N.D.S. score. Furthermore, no relationship was found between the anti-neuroblastoma cell antibodies and any clinical subset of neuropathy. This might be explained by the fact that the majority of our patients had a mixed sensorimotor neuropathy, with only small numbers suffering from a pure motor or sensory neuropathy. Similarly we were not able to relate the presence of the anti-neuroblastoma cell antibodies to axonal or demyelination changes because of the smaller number of patients with pure axonal features.

Anti-neuroblastoma cell antibodies have been used in the diagnosis of paraneoplastic syndromes such as the subacute sensory neuropathy and cerebellar degeneration which are associated with autoantibodies directed against various neural antigenic structures (14). In 1985 Graus et al. described an antibody reacting with neuronal nuclei, called anti-Hu, classically encountered in cases of Denny-Brown neuropathy (15). The Hu antigen, a pan-neuronal intranuclear antigen with a molecular weight of 35 to 40 kD, is present in most CNS neurons and in cell lines such as the neuroblastoma cell (16, 17).

Since serological responses to neuroblastoma cells occur in different pathological conditions, they may indicate reactivity with a heterogeneous group of neuroblastoma cell antigens. We found that there were differences in the immunofluorescent staining patterns of neuroblastoma cells incubated with sera from neuropathy patients and controls. No staining was found on the surface of neuroblastoma cells, but a strong cytoplasmic staining pattern was seen with the majority of the neuropathic patients’ sera tested. The staining pattern on neuroblastoma cells was totally different from the staining seen in synovial fibroblasts, which was predominantly nuclear or filamentous, and was only found with some sera. We interpret our results as suggesting that in peripheral neuropathy antibodies are developed which recognize particular neuroblastoma antigens, and that these are specific to neural cells and are not found on fibroblasts. Western blotting with different sera indicated that a heterogeneous group of antigens was being recognised in the neuroblastoma cells, and no particular bands were common to all patients. The reactivity of the blots and immunofluorescent staining were weak or absent with sera from healthy volunteers and RA controls, although the latter sometimes demonstrated nuclear staining.

The presence of an immunological response to neuroblastoma cell antigens among RA patients with PN might be the result of a shared homology between antigens of the neuroblastoma cell line (SK.N.SH) and peripheral nerve antigens. Absorption studies have demonstrated a partial homology between antigens of the neuroblastoma cell line 108cc15 and the human sciatic nerve (8). In contrast, the absence of an immunological response to neuroblastoma cells among some of the neuropathy patients may indicate the involvement of other factors in the pathogenesis of this condition. RA patients with neuropathy who develop antibodies against neuroblastoma cells may represent a distinct subset of the disease. For example, in myasthenia gravis a subset of patients develop anti-neuroblastoma cell antibodies which display a different pattern of regulation.

Table II. Results of immunofluorescence staining of neuroblastoma cells with sera from patients with RA neuropathy, RA controls and healthy volunteers.

<table>
<thead>
<tr>
<th>Stain intensity</th>
<th>RA neuropathy n = 18</th>
<th>RA controls n = 9</th>
<th>Healthy volunteers n = 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strong (3+)</td>
<td>13</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Moderate (2+)</td>
<td>5</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Weak or none (+/-)</td>
<td>0</td>
<td>4</td>
<td>6</td>
</tr>
</tbody>
</table>

Fig. 5. Western blotting of neuroblastoma antigens with sera from PN patients (lanes 1-6), RA controls (lanes 7 and 8) and a healthy control (lane 9). The sera from PN patients show a variety of banding patterns. Molecular weight markers in kDa are shown.
from that of the anti-acetylcholine receptor antibodies, including a reduction of anti-neuroblastoma cell antibody levels following thymectomy (18). In our study we were unable to detect any clinical differences between PN patients with or without these antibodies, although larger numbers of patients with different types of neuropathy would be needed to answer this question with any certainty.

At present, it is not clear if the presence of anti-neuroblastoma cell antibodies with a specific reactivity are associated with rheumatoid neuropathy only, or are common among other neuroarthropathies. Antibodies against the neuroblastoma cell may recognise a heterogeneous group of antigens and may arise subsequent to nerve damage. They may not, therefore, necessarily be pathogenic. Furthermore, because of the weak staining of neuroblastoma cells with some normal and non-neuropathic sera we cannot rule out the possibility that these are natural antibodies, normally found at low levels but elevated in certain disease states. It is also possible that some antibodies to neuroblastoma cells are the same as those previously detected by us against gangliosides and neurofilaments (5, 6). However, correlations with the levels of these antibodies were weak or absent, and different groups of PN patients were identified by the various antibodies, suggesting that different antigens are being recognised. A combination of these markers allowed the identification of > 70% of patients with PN, though it would not normally be practical to carry out so many ELISAs in the clinical setting. Larger studies of the clinical and neurophysiological characteristics of anti-neuroblastoma cell antibody-positive and negative patients will be required to establish if these particular antibodies reflect a distinct group of rheumatoid neuropathy, as well as to advance our understanding of its pathogenesis.

The role of vasculitis in the development of peripheral neuropathy is well established, particularly in mononeuropathies and multiplex, and we have shown recently that RA patients with PN have elevated levels of soluble adhesion molecules and anti-endothelial cell antibodies (AECA), which may result from endothelial cell activation and/or damage (19). This reinforces the view that the development of antibodies to neural antigens is likely to arise from the exposure of peripheral nerves to the immune system following vascular damage. However, such damage around the peripheral nerves may sometimes result in a non-systemic vasculitis which is not clinically apparent. The anti-neuroblastoma cell antibodies in this study did not appear to reflect vasculitis, because only 2 patients among the 13 with extra-articular features had significant levels of anti-neuroblastoma cell antibodies, while the rest exhibited levels similar to those seen in RA patients without vasculitis. Nonetheless, we found that the levels of IgM anti-neuroblastoma antibodies correlated with circulating E-selectin (r = 0.5, p = 0.01) levels, while IgG anti-neuroblastoma antibodies correlated with anti-endothelial cell antibodies (r = 0.42, p = 0.04). This suggests that the presence of anti-neuroblastoma antibodies may be associated with some endothelial cell activation or damage, but it remains to be established whether this is associated with subclinical vasculitis. Further studies are also needed to establish whether anti-neuroblastoma antibodies exhibit any cytotoxic activity against peripheral nerve cells, and which cross-reactive determinants, if any, are shared.

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References