Excessive nitric oxide (NO)/peroxynitrite (ONOO·) generation has been implicated in the pathogenesis of multiple sclerosis (MS). This hypothesis is supported by the demonstration of increased NO synthase (NOS) activity and NOS mRNA in astrocytes associated with demyelinating lesions in post mortem MS brain (1,2). Further evidence for increased NO/ONOO· formation comes from our observation that the concentration of nitrate + nitrite (degradation products of NO/ONOO·) was elevated by 70% in the cerebrospinal fluid of MS patients (3).

We have shown that exposure of astrocytes (a major source of NO within the brain) to interferon (IFN)-α+ lipopolysaccharide (LPS) results in increased NOS activity, a corresponding increase in NO release, and damage to the mitochondrial respiratory chain in the absence of cell death (4). While astrocytes appear particularly sensitive to NO/ONOO·, neurones appear particularly sensitive to NO/ONOO· (5). We hypothesise that increased formation of astrocytic NO occurs in the active phase of MS metabolically compromising neighbouring neurones.

IFN-β preparations are currently used in the treatment of MS, and we have recently shown that while exposure of astrocytes to IFN-γ (500 U/ml) results in stimulation of NOS activity and increased NO release, IFN-α/β (500 U/ml) has minimal effect on astrocytic NO formation (6). Furthermore pretreatment of astrocytes with IFN-α/β significantly prevented stimulation of NO release (p<0.05) by IFN-γ and IFN-γ + LPS, by approximately 65% and 38% respectively (7). Therefore we speculated that the ability of IFN-α/β to attenuate astrocytic NO production may be beneficial to neighbouring NO-sensitive cells.

This hypothesis was investigated using a coculture system (8) of mouse neurones and cytokine-stimulated astrocytes. Astrocytes were seeded onto culture inserts (0.4 mm membrane pore size) and treated for 24h as follows:

(1) Control
(2) IFN-γ (500 U/ml) + LPS (1 µg/ml)
(3) L-NAME (1 mM) + IFN-γ + LPS
(4) IFN-α/β (500 U/ml) for 24h then IFN-γ + LPS for 24h
(5) IFN-α/β alone (500 U/ml)

At the end of the incubation period, the media was removed, the filters washed and the astrocyte containing filters were transferred to wells containing neurones. These neuronal-astrocyte co-cultures were incubated for 24h. The astrocyte inserts were then removed and the neurones were trypsinised and pelleted. The activities of the mitochondrial complexes - NADH-CoQ (complex I), succinate-cytochrome c reductase (complex II/III) and cytochrome c oxidase (complex IV) - and citrate synthase (a mitochondrial marker) were determined in cell homogenates.

The exposure of neurones to IFN-γ + LPS-treated astrocytes produced a significant loss of both complex I/II and complex IV (Table 1). Both the NOS inhibitor, L-NAME, and IFN-α/β pretreatment prevented this damage. Complex I was unaffected.

Table 1. Neuronal mitochondrial complex activities

Each value represents mean ± SEM (n=5)
* - significantly different to control (p<0.05)

<table>
<thead>
<tr>
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<th>Complex I/II (nmol/min/mg)</th>
<th>Complex IV (k/min/mg)</th>
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<tr>
<td>Neurones alone</td>
<td>9.9 ± 0.7</td>
<td>0.97 ± 0.10</td>
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<tr>
<td>Neurones cocultured with astrocytes treated as follows:</td>
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<tr>
<td>Control (nothing)</td>
<td>12.0 ± 1.4</td>
<td>1.07 ± 0.11</td>
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<tr>
<td>IFN-γ + LPS</td>
<td>7.4 ± 1.3 (*)</td>
<td>0.75 ± 0.13 (*)</td>
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<tr>
<td>NAME + γ-LPS</td>
<td>11.1 ± 0.7</td>
<td>1.06 ± 0.11</td>
</tr>
<tr>
<td>αβ then γ-LPS</td>
<td>11.7 ± 1.1</td>
<td>1.01 ± 0.21</td>
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These results indicate that stimulation of astrocytic NOS activity causes significant damage to the mitochondrial activities of complexes I/II and IV of the co-cultured neurones. The inclusion of the NOS inhibitor, L-NAME, prevented this effect confirming that this damage is NO-mediated. Furthermore the ability of IFN-α/β pretreatment of astrocytes to prevent neuronal mitochondrial damage, confirms our previous observation that IFN-α/β attenuates the ability of astrocytes to produce NO upon subsequent stimulation. In view of these findings, we suggest that a possible mechanism of action of IFN-β in the treatment of MS is that it prevents astrocytic NO production and therefore limits damage to neighbouring NO-sensitive cells, such as neurones.

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