Investigation of possible cytokine induction in peripheral blood mononuclear cells by heat-stable serotypes of *Campylobacter jejuni*

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**ABSTRACT**

Several *Campylobacter jejuni* heat-stable (HS) serotypes have been associated with the autoimmune Guillain–Barré neurological syndrome (GBS). In order to examine the possible involvement of cytokines in this phenomenon, the levels of three pro-inflammatory cytokines (interleukin (IL)-2sRa, IL-6 and interferon (IFN)-c) and one anti-inflammatory cytokine (IL-10) were measured in peripheral blood mononuclear cells after induction by different *C. jejuni* serotypes. No differences were found for IL-6, IFN-c and IL-10, but the non-sialylated serotype HS:3 was associated with decreased production of IL-2sRa. The results raise the possibility that absence of sialylation might be associated with the inability to induce inflammatory factors such as cytokines.

**Keywords** Autoimmunity, *Campylobacter jejuni*, cytokine receptors, cytokines, Guillain–Barré syndrome

**Original Submission:** 12 January 2004; **Revised Submission:** 13 May 2004; **Accepted:** 8 June 2004

*Clin Microbiol Infect* 2005; 11: 63–65
10.1111/j.1469-0691.2004.01038.x

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Guillain–Barré Syndrome (GBS) may result from the production of antibodies to *Campylobacter jejuni* polysaccharides, which then cross-react with gangliosides of the peripheral nerves [1]. In addition, T-lymphocyte subsets, monocytes and cytokines can trigger immune-mediated inflammatory responses that cause demyelination [2,3]. Certain pro- and anti-inflammatory cytokines have been found in the peripheral blood of GBS patients [4], and several heat-stable (HS) serotypes (Penner serotypes) of *C. jejuni* are associated with GBS [5]. However, nothing is known about a link between particular serotypes and the production of cytokines, which could play a role in the pathogenesis of the disease. As a first step towards understanding the pathogenesis of GBS, the present study investigated the in-vitro induction of cytokines (interleukin (IL)-2sRα, IL-6, interferon (IFN)-γ and IL-10) by several HS serotypes of *C. jejuni*.

All *C. jejuni* strains were isolated from the stools of hospitalised patients and were identified with conventional bacteriological methods. Serotyping was performed with a set of 25 antisera (*Campylobacter Antisera ‘Seiken’ Set; Denka Seiken, Tokyo, Japan*) based on Penner’s HS serogroups. The selection of serotypes included in the study was based on the number of strains available in our collection, so that between five and seven strains belonging to each serotype could be used for cytokine induction. Seven serotypes tested (HS:2, HS:5, HS:19, HS:37, and the complexes HS:1,44, HS:4,13,16,43,50, HS:23,36,53) have been associated frequently with GBS; five serotypes (HS:8, HS:11, HS:15, HS:31, HS:52) have not yet been associated with GBS; and one serotype (HS:3) was the only serotype thought not to be capable of causing GBS [5].

Peripheral blood mononuclear cells (PBMCs) were obtained with the use of Biocoll separating solution (Biochrom, Berlin, Germany). Pooled blood was collected from healthy blood donors. Isolated cells were suspended in RPMI-1640 medium enriched with glutamate and heat-inactivated fetal calf serum 10% v/v (Biochrom) at a final concentration of between 5 x 10⁶ and 1 x 10⁷ cells/mL. A single-cell preparation was used for all the experiments.

In-vitro induction of cytokines in PBMCs by various *C. jejuni* strains was performed as described previously for *Streptococcus mutans* [6].

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Cells were incubated at 37°C, in a humidified CO2 5% v/v chamber, for 4 days. Preliminary kinetic studies indicated that cytokine levels were near maximal after 3 days. On day 4, cells were sedimented by centrifugation at 200 g at 4°C, after which the supernatants were stored at −80°C until assayed for cytokine levels. Aliquots were cultured before and after incubation, to detect any bacterial contamination. Five control experiments, consisting of PBMC suspensions without bacteria, were run in parallel. Levels of IL-2sRa, IL-6, IFN-γ and IL-10 in the culture supernatants were determined with human cytokine enzyme-linked immunosorbent assay (ELISA) kits (Quantikine; R&D Systems Inc., Minneapolis, MN, USA). Statistical analysis was performed with the Stata program v. 7 (University of California, Los Angeles, CA, USA), with use of the non-parametric Kruskal–Wallis and Wilcoxon tests because of the lack of normality.

The concentrations of the four cytokines in the supernatants of the PBMC suspensions after induction with different C. jejuni serotypes are shown in Table 1. Detectable levels were obtained in every case, but the values differed greatly from strain to strain. There were no significant differences in the levels of IL-6, IFN-γ and IL-10 between the C. jejuni serotypes and the controls, as calculated with the non-parametric Kruskal–Wallis test. For IL-2sRa, significant differences (p < 0.0003) were found with the Kruskal–Wallis test. Pairwise analysis with the non-parametric Wilcoxon test revealed significant differences (p < 0.05) between HS3 and the other serotypes and controls.

### Table 1. Cytokine levels induced by different Campylobacter jejuni serotypes (1.5 × 10^8 CFU/mL) in supernatant fluids from peripheral blood mononuclear cells (PBMCs)

<table>
<thead>
<tr>
<th>Serotype (no. of strains)</th>
<th>Mean ± SD (pg/mL) in 10^8 PBMC/mL supernatant</th>
<th>IL-2sRa</th>
<th>IL-6</th>
<th>IFN-γ</th>
<th>IL-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (5)</td>
<td>21.46 ± 10.96</td>
<td>88.30 ± 52.61</td>
<td>41.98 ± 30.80</td>
<td>49.27 ± 39.19</td>
<td>42.60 ± 30.70</td>
</tr>
<tr>
<td>HS1,14 (6)</td>
<td>31.67 ± 3.33</td>
<td>40.36 ± 39.19</td>
<td>49.27 ± 39.19</td>
<td>42.60 ± 30.70</td>
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<tr>
<td>HS2 (5)</td>
<td>11.04 ± 2.59</td>
<td>54.18 ± 36.84</td>
<td>50.28 ± 62.92</td>
<td>100.00 ± 172.51</td>
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</tr>
<tr>
<td>HS3 (5)</td>
<td>2.17 ± 1.87</td>
<td>22.08 ± 12.81</td>
<td>32.42 ± 53.04</td>
<td>147.40 ± 208.19</td>
<td></td>
</tr>
<tr>
<td>HS4,13,16,43,50 (6)</td>
<td>13.75 ± 9.76</td>
<td>24.02 ± 13.98</td>
<td>104.83 ± 201.81</td>
<td>38.23 ± 54.68</td>
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</tr>
<tr>
<td>HS5 (7)</td>
<td>13.92 ± 6.86</td>
<td>41.05 ± 30.66</td>
<td>37.11 ± 37.78</td>
<td>143.22 ± 176.77</td>
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</tr>
<tr>
<td>HS6 (6)</td>
<td>17.92 ± 4.77</td>
<td>41.25 ± 23.80</td>
<td>5.15 ± 11.53</td>
<td>23.80 ± 53.21</td>
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<tr>
<td>HS7 (5)</td>
<td>17.58 ± 4.25</td>
<td>33.08 ± 25.62</td>
<td>23.56 ± 37.51</td>
<td>39.66 ± 54.99</td>
<td></td>
</tr>
<tr>
<td>HS9 (5)</td>
<td>18.59 ± 7.27</td>
<td>19.34 ± 6.84</td>
<td>0.26 ± 0.59</td>
<td>49.40 ± 98.11</td>
<td></td>
</tr>
<tr>
<td>HS23,36,53 (6)</td>
<td>15.52 ± 2.59</td>
<td>16.76 ± 13.18</td>
<td>32.19 ± 54.48</td>
<td>94.56 ± 146.55</td>
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<tr>
<td>HS31 (7)</td>
<td>9.32 ± 4.73</td>
<td>49.63 ± 46.83</td>
<td>42.11 ± 57.71</td>
<td>6.57 ± 17.38</td>
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<tr>
<td>HS37 (6)</td>
<td>13.05 ± 6.47</td>
<td>12.59 ± 7.46</td>
<td>7.90 ± 16.77</td>
<td>0.23 ± 0.33</td>
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<tr>
<td>HS52 (6)</td>
<td>13.46 ± 2.74</td>
<td>41.91 ± 43.95</td>
<td>34.24 ± 36.67</td>
<td>24.00 ± 27.45</td>
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</table>

In GBS, it is known that IL-6, synthesised by activated CD4+ cells of the Th2 phenotype, promotes the production of antibodies against peripheral nerve sheath myelin components [7]. High IL-6 concentrations have been found in active GBS, and have been correlated with the clinical signs of the disease [3,8]. However, no correlation has been observed between cerebrospinal fluid IL-6 levels and other disease variables, or between cerebrospinal fluid and serum IL-6 levels [9]. In the present study, IL-6 production by induced PBMCs was similar for the various C. jejuni serotypes tested and did not differ from the levels in controls.

IFN-γ is produced in increased amounts by activated T-cells in the blood of GBS patients [10], but no differences in IFN-γ plasma levels have been observed between GBS patients and healthy individuals [3,11]. Similarly, no in-vitro differences in IFN-γ induction were found between GBS-triggering or GBS-non-triggering C. jejuni serotypes and the controls.

With respect to IL-10, no detectable levels have been found in the serum or cerebrospinal fluid of GBS patients [12]. In the experimental model used in the present study, IL-10 showed great intra- and inter-serotype variation, but no significant correlation could be established.

IL-2 is produced predominantly by CD4+ Th1 cells. The soluble form of the IL-2 receptor (IL-2sRa) appears in serum, as it is highly expressed by cells [13]. It has been suggested that a correlation exists between the IL-2sRa level in serum and the activity of autoimmune diseases [14]. In GBS, high levels of IL-2sRa correlate with disease severity, while low concentrations parallel clinical improvement, suggesting a role for this cytokine in the pathogenesis of GBS [9,15]. Increased levels of IL-2 in GBS would enhance T-cell activation and stimulate B-cells to secrete autoantibodies and to produce other proinflammatory cytokines, which may injure the myelin sheath and/or Schwann cells. However, increased induction of IL-2sRa by the GBS-associated or GBS-non-associated C. jejuni serotypes was not observed, with the exception of HS3, which induced 5–10-fold lower levels of IL-2sRa.

The pathogenesis of GBS, associated with antecedent C. jejuni infection, is based on antibody cross-reactions between sialylated peripheral nerve gangliosides and the sialylated lipopolysaccharides of C. jejuni [1]. The lipopolysaccharide...
of HS:3 is not sialylated [9,16] and it does not mimic human gangliosides [17], and this serotype has not been associated with antecedent infection in GBS patients. Lipopolysaccharide from HS:3 has been used as a negative control in experiments with anti-ganglioside antibodies [18] and, in the present study, HS:3 failed to induce significant IL-2sRa levels when compared to the other serotypes. Based on these results, the question arises as to whether the non-sialylated structure of HS:3 may be related, via mechanisms involving IL-2sRa induction, to its inability to cause GBS. While the absence of molecular mimicry with respect to human peripheral nerve gangliosides could also be responsible, further studies at the cellular and molecular level are clearly required to improve our understanding of the delicate interactions among immunoregulatory mechanisms, and to enable the possible development of new therapeutic approaches.

ACKNOWLEDGEMENTS

We thank B. Pantelis for performing the statistical analyses.

REFERENCES


