Circulating tumour necrosis factor alpha & soluble TNF receptors in patients with Guillain-Barre syndrome

V.V. Radhakrishnan, M.G. Sumi, S. Reuben, A. Mathai & M.D. Nair*

Departments of Pathology & *Neurology, Sree Chitra Tirunal Institute for Medical Sciences & Technology (SCTIMST), Thiruvananthapuram, India

Received October 21, 2002

Background & objectives: Tumour necrosis factor-α (TNF-α) is regarded as one of the immune factors that can induce demyelination of peripheral nerves in patients with Guillain-Barre syndrome (GBS). This present study was undertaken to find out the role of TNF-α and soluble TNF receptors in the pathogenesis of GBS; and to study the effect of intravenous immunoglobulin (ivIg) therapy on the serum TNF-α and soluble TNF receptors in patients with GBS.

Methods: Thirty six patients with GBS in progressive stages of motor weakness were included in this study. The serum TNF-α and soluble TNF receptors (TNF-RI, TNF-RII) were measured in the serum samples of these patients before and after ivIg therapy by a sandwich ELISA.

Results: Of the 36 patients with GBS, 26 (72.2%) showed elevated serum TNF-α levels prior to ivIg therapy. Following a complete course of ivIg therapy there was a progressive decrease in the serum TNF-α concentrations in these 26 patients. On the other hand, the soluble TNF receptors, particularly TNF-RII showed an increase in the serum of GBS patients following ivlg therapy.

Interpretation & conclusion: The results indicate that ivIg reduces the serum TNF-α concentrations in the GBS patients having elevated levels prior to ivIg therapy. Elevated serum levels of soluble TNF receptors following ivlg therapy may play a protective role by inhibiting the demyelinating effect of TNF-α in the peripheral nerves of patients with GBS.

Key words Clinical recovery - Guillain-Barre syndrome - immunoglobulins ivIg - tumour necrosis factor receptors (sTNF-RI, sTNF-RII) - tumour necrosis factor alpha (TNF-α)

Guillain-Barre syndrome (GBS) is an acute inflammatory demyelinating polyneuropathy, clinically characterised by areflexia, and progressive flaccid paralysis of both extremities. The precise etiopathogenesis of the disease is not well defined and it is generally regarded that GBS is an immune-mediated demyelinating disease. Activated T- lymphocytes and macrophages are the principle source of cytokines including tumour necrosis factor-alpha (TNF-α) - a primary mediator of inflammation. TNF-α is known to induce demyelination of the nervous system1. It has also been reported that TNF-α is capable of inducing selective and specific damage to myelin in vitro2 and this may eventually lead to degeneration of the axons. Elevated serum levels of TNF-α have been documented as well as incriminated in the pathogenesis of GBS3-6. However, TNF-α alone cannot be implicated in the pathogenesis of GBS and other immune factors such as myelin associated
glycoproteins as well as antibodies against ganglioside have also been implicated in the pathogenesis of GBS\textsuperscript{7,8}.

TNF-\(\alpha\) mediates its biological effect by interacting with two distinct TNF receptors, TNFR-p55 (sTNFR-I) and TNFR-p75 (sTNFR-II)\textsuperscript{9}. Both of these receptors exist in soluble form (sTNFR) as well as in cell surface membrane-bound form (TNFR). The soluble form binds with TNF-\(\alpha\) and in higher concentrations can act as inhibitors of the biological activity of TNF-\(\alpha\)\textsuperscript{10}. However, in lower concentrations, the soluble TNF receptors can prolong the biological half-life of TNF-\(\alpha\) activity\textsuperscript{11}. Regulation of TNF receptor shedding represents a sensitive mechanism controlling TNF-\(\alpha\) availability. Disturbances in the TNF receptor shedding might be linked with TNF-\(\alpha\) mediated demyelinating autoimmune diseases of the central nervous system including GBS.

During the past decade, intravenous Ig has been widely used in the management of several inflammatory demyelinating diseases of the CNS including GBS. Despite this, the precise immunomodulatory mechanism of action of ivIg is not well understood. To gain additional insight into these immunological mechanisms, the present study was undertaken to estimate the serum concentrations of TNF-\(\alpha\) and the soluble TNF receptors (sTNFR-I, sTNFR-II) in patients with GBS before and after the completion of ivIg therapy; and to assess the relationship between TNF-\(\alpha\), soluble TNF receptors and their role in the pathogenesis and prognosis of GBS.

**Material & Methods**

**Patients:** This prospective study (September 2001 to August 2002) was conducted at the Sree Chitra Tirunal Institute for Medical Sciences and Technology (SCTIMST), Thiruvananthapuram, India - a major tertiary referral center for Neurological diseases in Kerala state. Thirty six patients (21 males and 15 females) who fulfilled the diagnostic criteria for GBS\textsuperscript{12} were studied over a period of 12 months. The age of these patients ranged between 6-68 yr (mean age 34±8.5 yr). Most of the patients (28/36) gave a history of upper respiratory tract infection within three weeks (median 16 days), prior to the onset of neurological illness. In 8 patients, no predisposing factors were present. None of the 36 patients had any associated systemic diseases such as diabetes or hypertension. At the time of admission, the disease severity was assessed by the clinical scale\textsuperscript{13}. Stage I: minor symptoms and signs but capable of manual work (n=0); stage II: able to walk without support (n = 4); stage III: able to walk with support (n=14); stage IV: confined to chair or bed (n= 12); stage V: requiring assisted ventilation (n = 6). All these patients were referred to SCTIMST for specific management. The duration of hospital stay ranged between 14-45 days. Standard intravenous immunoglobulin (ivIg) therapeutic protocol approved by the ethics committee of this institute as a therapeutic measure for the management of patients with GBS was used. Informed consent was obtained from all the patients prior to the therapy. However consent for nerve biopsy could not be obtained in any one of these patients. Following admission, patients received ivIg (400 mg/kg body wt/day) for five consecutive days. Serum samples were collected before and after the ivIg therapy, as well as at the time of discharge from the hospital from all these patients.

**Control subjects:** Control serum samples were obtained from two groups. The disease controls included 36 age-matched patients with other neurological illness including diabetic peripheral neuropathy (n=20); paralytic rabies (n=3); myeloradiculopathy (n = 4); transverse myelitis (n=4); and hereditary sensory motor neuropathy (n = 5). Serum samples were also collected from 36 age-matched voluntary blood donors (healthy control) who had no neurological illness in the recent past or chronic illness.

**Sample preparation:** Venous blood samples (4-6 ml) were collected in sterile glass tubes and were allowed to clot spontaneously over 1 h. Serum was collected by centrifugation and filtered through 0.22\(\mu\) disposable sterile filter (Millipore) to remove particulate material such as fibrin. All samples were coded and frozen at -70\(^\circ\)C until the time of assay. Repeated thawing and refreezing was avoided to the maximum extent possible. Protease inhibitor
aprotinin (Sigma, USA) 10,000 kallikerin inhibitory units/ml were added to all the sera to prevent protein degradation.

**TNF-α assay**: A sandwich ELISA, (using TNF-α immunoassay kits, Sigma, USA) was used to determine the serum levels of TNF-α in the test and control subjects. The assay was performed according to the manufacturer’s instructions. The lowest serum TNF-α detection limit was 4.4 pg/ml. A standard was run on each plate, using the recombinant human TNF-α standard in serial dilution (1000 to 0 pg/ml). The serum TNF-α concentrations in the patients with GBS before and after the ivIg treatment as well as at time of their discharge from the hospital were assayed. Coded serum samples were assayed on two different occasions to evaluate the reproducibility of the assay. Reproducibility of the assay was obtained by the Pearson’s correlation coefficient of +0.8.

**sTNF-RI, sTNF-RII assays**: sTNF- RI and sTNF- RII levels in the sera of GBS and control groups were measured by the commercial ELISA immunoassay kits (Sigma, USA). ELISA was performed according to the manufacturer’s instructions. Prior to estimation the assay was standardised using human sTNF- RI and sTNF- RII as standards as well as highly specific monoclonal antibodies for sTNF-RI and sTNF-RII. A standard graph was obtained. The lowest serum detection limit for sTNF-R1 and sTNF-RII were 300 pg/ml and 400 pg/ml respectively.

**ELISA for IgG antibodies to ganglioside**: An indirect ELISA was performed to detect IgG antibody titres in the sera of GBS and control groups with the commercially available GM1, GM2, CD1a, GD1b gangliosides (Sigma, USA). Serum samples with an antibody titre >1:320 were regarded as positive in the ELISA.

For statistical analysis Mann-Whitney and Student’s ‘T’ test were used to compare the data in GBS and control groups. Correlation between neurological recovery and TNF-α. levels was calculated using Pearson correlation method.

**Results**

Patients were referred to this hospital 2-16 days after the onset of motor weakness (median 8 days). At the time of admission (day-1) prior to the ivIg therapy, all 36 patients were in the progressive stages of motor weakness. Following a complete course of ivIg therapy, 26 patients who had elevated serum TNF-α levels prior to ivIg therapy manifested clinical improvement in their motor functions and at the time discharge from the hospital (14-28 days; median 21 days) they showed definite signs of clinical recovery. All the patients in stages II (n=4) and III (n=14) showed complete neurological recovery. Eight of the 12 patients in stage IV were able to walk without support and the remaining 4 patients were able to walk with support but they were confined to chair. Four patients in stage V did not recover neurologically and they were still confined to bed at the end of the study and required ventricular support. Two patients developed respiratory infection and expired during the hospital stay. Thus 10 GBS patients (4 of stage IV and 6 of stage V) did not recover neurologically following ivIg therapy. The electrophysiological studies in these 10 patients indicated axonal changes greater than demyelination. This would be a reason for the poor response to ivIg therapy in these patients.

**Serum TNF-α levels**: In all healthy controls and in 34 of 36 patients in the disease control group the serum TNF-α levels were < 4.4 pg/ml. In two patients in the disease control group (chronic inflammatory demyelinating polyneuropathy) serum TNF-α levels were 27.5 and 30.5 pg/ml respectively. A cut-off was chosen at 31 pg/ml in the ELISA to score a test positive. Accordingly 26 of the 36 (72.2%) GBS patients (Table) had elevated serum TNF-α which showed a progressive decrease following ivIg therapy. At the time of discharge a positive correlations was seen between neurological recovery and decreasing serum TNF-α concentrations in these patients (correlation coefficient value ‘r’ >0.7). In the remaining 10 GBS patients, the serum TNF-α levels ranged between 4.5-38.5 pg/ml (mean 18.5±4.5 pg/ml) with significantly elevated IgG antibody titres (>320) to GD1b ganglioside. In the 26 patients with elevated serum TNF-α levels the antibody titres to GD1b ganglioside ranged between 1:20 to 1:160.
were < 300 pg/ml and < 400 pg/ml respectively. In GBS patients prior to ivIg therapy, the serum sTNF-RI, sTNF-RII levels were not significantly elevated. However there was a gradual increase in the serum concentrations of sTNF-RI, sTNF-RII in 26 of 36 GBS patients following ivIg therapy. sTNF-RI levels showed an increase between 10-14 days of hospitalization (375-490 pg/ml; mean 425±46 pg/ml) and remained more or less static till the time of discharge from the hospital. However serum sTNF-RII levels showed a significant increase (820-2150 pg/ml; mean 1250±105 pg/ml) between 14-28 days of hospitalization. At the time of discharge from the hospital, the serum sTNF-RII levels were significantly ($P$ <0.05) higher than sTNF-RI. The ratios between TNF-α and TNFR-I, TNFR-II showed a decrease (Table) following ivIg therapy. This would indicate a reduction in the unbound TNF-α in the sera of patients with GBS following ivIg therapy.

### Discussion

This study indicated that 72.2 per cent GBS patients at the time of admission had elevated serum TNF-α and this was similar to that reported earlier$^{3-5}$. The data in this study also emphasized that elevated serum TNF-α levels in patients with GBS decreased following ivIg therapy and there was a positive correlation between the neurological recovery and decreasing serum levels of TNF-α in these patients at the time of discharge from the hospital. The finding of increased serum levels of soluble TNF receptors during the recovery phase of the disease has earlier been described in patients with GBS$^{14,15}$. Creange $et$ $al^{14}$ analyzed 24 GBS patients following plasma exchange and observed a sustained increase of soluble TNF receptor sTNF-RI (p55) during the recovery phase of the disease. We found a sustained and significant increase in the TNF-RII in the sera of GBS patients following ivIg therapy. Sharief $et$ $al^{15}$ also reported high serum concentrations of soluble TNF receptors during the recovery phase of the disease following ivIg therapy. They calculated the ratio between serum TNF-α and soluble TNF receptors and observed that the clinical improvement in their patients correlated with a reduction in the unbound TNF-α during the active phase of the disease. In the present study we also observed a reduction in the unbound TNF-α. It needs to be emphasized that elevated TNF-α alone may not be responsible for the pathogenesis in all patients with GBS. Other circulating immune factors such as other cytokines (interleukin) and antibodies to ganglioside, may also play a role. Campylobacter jejuni can also play a role in the pathogenesis of the disease$^7$. In our data, 10 of 36 GBS patients had significantly elevated serum antibody titres to GD1b ganglioside.

Several immunomodulatory mechanisms of action of ivIg have been described. Sharief $et$ $al^{15}$

---

**Table.** Serum TNF-α, sTNF-RI, sTNF-RII concentrations in patients with GBS before and after intravenous ivIg therapy

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Before ivIg</th>
<th></th>
<th>After ivIg</th>
<th></th>
<th>At discharge</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Mean±SD</td>
<td>Range</td>
<td>Mean±SD</td>
<td>Range</td>
<td>Mean±SD</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>35-182</td>
<td>85±10.5</td>
<td>9-48.5</td>
<td>20.5±5.5*</td>
<td>11.5-42.5</td>
<td>20.6±5.5</td>
</tr>
<tr>
<td>TNFR-I (pg/ml)</td>
<td>310-350</td>
<td>325±21</td>
<td>375-490</td>
<td>425±46*</td>
<td>375-510</td>
<td>410±50</td>
</tr>
<tr>
<td>TNFR-II (pg/ml)</td>
<td>410-480</td>
<td>440±35</td>
<td>820-2150</td>
<td>1250±105*</td>
<td>850-2200</td>
<td>1290±110</td>
</tr>
<tr>
<td>TNF-α: TNFRI</td>
<td>0.23</td>
<td></td>
<td>0.014</td>
<td></td>
<td>0.048</td>
<td></td>
</tr>
<tr>
<td>TNF-α: TNFRII</td>
<td>0.19</td>
<td></td>
<td>0.016</td>
<td></td>
<td>0.015</td>
<td></td>
</tr>
</tbody>
</table>

No. of GBS patients with elevated TNF-α prior to ivIg therapy =26/36; *$P$ <0.05 compared to before ivIg therapy; serum TNF-RI in control groups < 300 pg/ml; serum TNF-RII in control groups < 400 pg/ml

TNF-α, tumour necrosis factor - α; sTNF-RI, soluble tumour necrosis factor receptor-I; sTNF-RII, soluble tumour necrosis factor receptor-II; GBS, Guillain-Barre syndrome; Ig, immunoglobulin
considered that the sustained decrease in the serum cytokine levels following ivIg therapy in patients of GBS would suggest a down-regulation of cytokine production. They also observed elevated serum levels of soluble TNF receptors following ivIg therapy in GBS patients and this could possibly indicate continuous release of soluble TNF receptors during the recovery phase of the disease. Thus divergent kinetics of TNF-α and soluble TNF receptors during the recovery phase of the disease in the present study would suggest a spontaneous decrease of the inflammatory reaction within the involved nerves in patients with GBS. This may be a result of secondary down-regulation of TNF-α production due to ivIg therapy as well as to the sustained buffering effect of sTNFR particularly sTNF-II. Thus it may be concluded that elevated levels of soluble TNF receptors particularly TNF-RII are likely to influence the clinical course of GBS and appear to play a protective role as an inhibitor to the demyelinating effects of TNF-α. Prospective histopathological studies of the involved nerves in patients with GBS are essential to substantiate the protective role of soluble TNF receptors in GBS patients following ivIg therapy.

Acknowledgment

The authors acknowledge the Department of Biotechnology, New Delhi for financial support for this study.

References


Reprint requests : Dr V.V. Radhakrishnan, Professor & Chairman, Department of Pathology, Sree Chitra Tirunal Institute for Medical Sciences and Technology (SCTIMST), Thiruvananthapuram 695011, India