Circulating transforming growth factor beta 1 (TGF- β 1) in Guillain-Barré syndrome: decreased concentrations in the early course and increase with motor function

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Received 16 July 1997
Accepted 29 August 1997

Abstract

Objective—To delineate the possible implication of the immunosuppressive cytokine transforming growth factor beta 1 (TGF- β 1) in the pathogenesis of Guillain-Barré syndrome. Guillain-Barré syndrome is a disorder that may implicate cytokines in its pathogenesis. TGF- β 1 is a potent anti-inflammatory cytokine occasionally shown to be regulated in the course of demyelinating disorders.

Methods—The study measured circulating proinflammatory and anti-inflammatory cytokines from the progressing phase to early recovery in patients with Guillain-Barré syndrome. Plasma concentrations of TNF- α , IL-1 β , IL-2, IL-4, IL-6, IL-10, and TGF- β 1 were prospectively evaluated in 15 patients with Guillain-Barré syndrome every three days for the first 15 days after admission to hospital, and in 15 controls with non-inflammatory neurological diseases.

Results—Concentrations of TGF- β 1 in plasma were decreased in 13/15 patients (87 %) at day 1, remained low during progression and the plateau of paralysis (days 1–10), and then progressively increased up to control concentrations during early recovery (days 12–15). Concentrations of plasma TGF- β 1 correlated positively with motor function, the lowest values being found in the most disabled patients. Concentrations of plasma TGF- β 1 were decreased before any treatment, and during treatment by either plasma exchange or intravenous immunoglobulins, plasma exchange being associated with a more pronounced decrease in TGF- β 1 at day 7. Circulating TNF- α concentrations were raised, as previously reported, when other cytokines were either randomly increased (IL-2, IL-6), or undetectable (IL-1, IL-4, IL-7, IL-10).

Conclusions—Down regulation of TGF- β 1 in the early course of Guillain-Barré syndrome could participate in neural inflammation.

(J Neurol Neurosurg Psychiatry 1998;64:162–165)

Keywords: cytokine; demyelinating neuropathy; Guillain-Barré syndrome; transforming growth factor β -1

Guillain-Barré syndrome is associated with circulating antibodies to glycoconjugates,¹ activated T lymphocytes in blood and peripheral nerve,² and activation of both resident and recruited macrophages.³ It is possible that systemically and locally released cytokines are important in the pathogenesis of Guillain-Barré syndrome. Raised serum concentrations of interleukin (IL)-2,⁴ IL-2 receptor,⁵ IL-6,⁶ and TNF- α ^{7–9} were found in patients with inflammatory demyelinating neuropathies. TNF- α is a major proinflammatory cytokine that could be implicated in early breakdown of the blood-nerve barrier, upregulation of endothelial adhesion molecules, a prerequisite for leucocyte trafficking to nerve tissue, macrophage activation, and myelin damage.¹⁰ Transforming growth factor beta 1 (TGF- β 1), a potent immunosuppressive molecule that antagonises the effects of TNF- α , IL-1, IL-2, and IFN- γ ¹¹ was previously considered instrumental in the healing process in Guillain-Barré syndrome, as it was found to be increased at the time of recovery in the circulation of patients with Guillain-Barré syndrome^{12–13} and in nerve of animals undergoing experimental allergic neuritis (EAN).¹⁴ The present study was triggered by previous evidence that downregulation of TGF- β 1 may take part in active inflammatory processes. Decreased production of TGF- β 1 was previously documented in patients with inflammatory demyelinating disorders, such as multiple sclerosis at the time of relapses,¹⁵ and POEMS syndrome,¹⁶ and experiments in TGF- β 1 null mice have shown that suppressed TGF- β 1 production is associated with severe tissue inflammation.^{17–18} In the present study attention was focused on circulating TGF- β 1 concentrations before recovery of Guillain-Barré syndrome—that is, at the time of progression of paralysis and plateau that precedes the switch to recovery.

Patients and methods

PATIENTS

Fifteen patients fulfilling clinical and electrophysiological diagnostic criteria for demyelinating Guillain-Barré syndrome¹⁹ were prospectively included in the study. Exclusion criteria for blood evaluation included fever, shock, and overt infection. Severity of disease was scored at the time of each blood sampling using the following scale: (A) able to walk $\geq$ 10 m with or without assistance; (B) bedridden or chairbound; (C) requiring assisted ventilation

Table 1 Classification of patients according to phase of the disease

	Progression	Plateau	Recovery
Day 1	14	1	0
Day 3	8	7	0
Day 7	5	8	2
Day 10	2	6	7
Day 12	1	3	11
Day 15	1	1	13

for at least part of the day. This staging is used by the French Cooperative Group on Plasma Exchange in Guillain-Barré syndrome.²⁰ In this classification, stage A corresponds to stage 2 and 3, stage B to stage 4, and stage C to stage 5 of the London classification.²¹

The patients were referred 4.8 (SD 3.9) days after onset of motor deficit. No patient had diarrhoea as a preceding event suggestive of *Campylobacter jejuni* infection. Blood was collected, at days 1, 3, 7, 10, 12, and 15 of hospital stay. Fourteen patients were in the progression phase of paralysis at day 1 of hospital stay, 13/15 were in the recovery phase at day 15, and a continuum from ending of progression to early recovery was seen from day 3 to day 12 (table 1). Patients were not treated at the time of first sampling, and blood was subsequently collected before plasma exchanges (PEs) or intravenous immunoglobulins (IVIgs). Ten patients were treated with four PEs from day 2 to 12 and the remaining five received IVIg (0.4 g/kg) from day 2 to 6. At day 1, seven patients were at stage A and eight at stage B; at day 15, four patients were at stage A, eight were at stage B, and three were at stage C.

Blood sampling was performed at 8 00 am, in EDTA tubes to avoid release of TGF- β 1 from platelets at the time of clot formation.²² Plasma was obtained by centrifugation within one hour of removal (10 minutes at 2500 rpm), and samples were kept frozen at -80°C until analysis.

CYTOKINE MEASUREMENTS

Circulating concentrations of cytokines were determined in all samples by enzyme linked immunosorbent assays (ELISAs). Total TGF- β 1 was evaluated in duplicate after acidification with 1N HCL (Genzyme, Cambridge, USA). The detection limit was 0.05 ng/ml. Both standard curve linearity ($r > 0.99$) and interassay coefficient of variation ($< 8\%$) were excellent.

Other evaluated molecules comprised proinflammatory cytokines: TNF- α (Immunotech, Marseille, France), IL-1 β (Immunotech, Marseille, France), IL-2, and IL-6 (Genzyme, Cambridge, USA); the anti-inflammatory cytokines: IL-4 and IL-10 (Genzyme, Cambridge, USA); IL-7 (R&D systems, Minneapolis, USA), a cytokine known to decrease TGF- β 1 production in vitro,²³ and cortisol.

CONTROLS

Control values were measured in plasma of 10 healthy subjects (normal controls) and 15 patients with neurological diseases (neurological controls), including old stroke deficit, non-obstructive hydrocephalus, degen-

erative dementia, and polyneuropathy due to vitamin deficiency.

STATISTICAL ANALYSIS

Cytokine concentrations were expressed as mean (SEM). The Mann-Whitney U test was used for comparison of average concentrations, and for correlations between TGF- β 1 concentrations and disease severity. $p < 0.05$ was considered significant. The Kruskal Wallis non-parametric analysis of variance (ANOVA) test was used for comparison of cytokine concentration evolution from day 1 to day 15.

Results

TGF- β 1 PLASMA CONCENTRATIONS

At day 1 of admission to hospital, plasma concentrations of TGF- β 1 (ng/ml) were below control values in 13/15 patients (87%). The mean TGF- β 1 plasma concentration decreased from admission (31.2 (7.7)) to day 3 (25.5 (5)), remained low from day 3 to day 10 (day 7: 28.2 (7.2); day 10: 30.4 (7.3)), and then progressively increased to control values at day 15 (day 12: 41.9 (7.1); day 15: 59.5 (10.5), fig 1). As a whole, plasma concentrations significantly increased from day 1 to day 12 ($p < 0.07$) and day 15 ($p < 0.03$).

Both patients treated by PE and patients treated by IVIg had lower TGF- β 1 plasma concentrations than neurological and healthy controls before and during treatment (day 3-day 10), and showed increasing TGF- β 1 plasma concentrations from day 1 to day 15 (IVIg day 1: 14.4 (2.2); day 15: 50.1 (16.5); $p < 0.03$; PE: day 1: 38.6 (11); day 15: 55.5 (9.8); $p = 0.08$). Patients treated by PE had significantly lower values than those receiving IVIg at day 7 (table 2).

TGF- β 1 plasma concentrations paralleled motor function as assessed by the disability score, the lowest values of TGF- β 1 being found in the most disabled patients: stage A (52.0 (7.3)), stage B (36.6 (4.7)), and stage C (23.2 (3.6)) ($p < 0.05$, fig 2).

OTHER CYTOKINES AND CORTISOL

TNF- α plasma concentrations (pg/ml) were raised in 9/15 patients (60%) at day 1, and 4/14 patients (29%) at day 15. Mean plasma

Figure 1 Concentrations of transforming growth factor-beta 1 (TGF- β 1) in plasma from day 1 to day 15, in Guillain-Barré syndrome represented as mean (SEM). HS=healthy subjects. NC=neurological controls.

Table 2 Concentration of TGF- β 1 (ng/ml) in plasma in patients with Guillain-Barré syndrome treated by IVIGs or plasma exchange

	IVIGs (n=5)		Plasma exchange (n=10)
Day 3	37.1 (10.6)	NS	17.9 (11.6)
Day 7	46.3 (10.6)	p<0.05	21.3 (8.1)
Day 10	43.8 (16)	NS	22.7 (7.1)
Day 3 to 10	39.3 (6.2)	p<0.05	22.7 (3.8)

Values are mean (SEM).

Figure 2 Concentrations of TGF- β 1 in plasma relative to the severity of the disease represented as mean (SEM). HS=healthy subjects. NC=neurological controls.

concentrations of TNF- α were not different at day 1 (15.9 (5.3)) and at day 15 (10.9 (5)). IL-1 β was detected in one patient, IL-2 in seven, IL-6 in five, IL-4 in two, and IL-10 in two. IL-7 was not detected.

Plasma cortisol concentrations (ng/ml) were above neurological control values (162.8 (19.3)) in 7/15 patients at day 1. Mean cortisol concentrations decreased from day 1 (214 (25.2)) to day 7 (143.3 (19.3)) (p<0.05), and then stabilised (day 10: 143.5 (16.3); day 15: 158.5 (8.3)). The highest values of cortisol were found in the most disabled patients: stage A (120.4 (10.7)), stage B (162 (11.7)), and stage C (197.8 (16.6)) (p<0.05).

Discussion

In the present study, concentrations of TGF- β 1 in plasma were decreased in 87% of patients with Guillain-Barré syndrome on admission, increased with motor function up to control concentrations during the next 15 days at the time of early recovery, and correlated well with the disability score. TNF- α concentrations were raised in 60% of patients on admission, and did not significantly decrease from day 1 to day 15 of the hospital stay. Other cytokines were either randomly increased (IL-2, IL-6), or undetectable in the circulation (IL-1, IL-4, IL-7, IL-10).

Treatments could not account for the down regulation of TGF- β 1 seen on admission of patients. During treatment, both patients undergoing PE and patients receiving IVIG had decreased plasma concentrations of TGF- β 1. PE was associated with lower concentrations of TGF- β 1 in plasma than IVIG at day 7. This could have resulted from some removal of TGF- β 1 (PE), or addition of TGF- β 1 (IVIG) at the time of treatment.

Direct down regulation of TGF- β 1 by TNF- α has not been documented to our knowledge. By contrast, TNF- α is a potent

activator of the hypothalamus-pituitary-adrenal axis²⁴ and corticosteroids may modulate TGF- β 1 production,^{25, 26} whereas the α 2-macroglobulin-TGF- β 1 complex inhibits steroidogenesis.²⁷ Increased circulating TNF- α concentrations were found in our patients, as previously reported,^{7, 9} and were initially associated with increased concentrations of cortisol when TGF- β 1 circulating concentrations were low. Interleukin-7, a cytokine known to down-regulate TGF- β 1,²³ and to upregulate IL-1, IL-6, IL-8, and TNF- α ,²⁸ was not detected in the circulation of our patients, suggesting that IL-7 production was either absent, or restricted to the tissue compartment, or had occurred transitionally before admission of patients.

According to previous human and experimental studies, both peripheral blood mononuclear cells¹⁵ and intraneural cells, including macrophages, lymphocytes, and possibly Schwann cells²⁹ may be sources of TGF- β 1 in inflammatory demyelinating disorders. TGF- β 1 mediates T cell suppression,^{30, 31} decreases endothelial cell adhesiveness of T lymphocytes,³² deactivates macrophage by suppressing the production of superoxide and nitric oxide,^{33, 34} and downregulates IFN- γ induced MHC class II expression on human cell lines.³⁵ Administration of recombinant TGF- β can mitigate experimental allergic neuritis (EAN)³⁶ and abrogate experimental allergic encephalitis (EAE).³⁷ Decreased plasma concentrations of TGF- β 1 early in the course of Guillain-Barré syndrome can be interpreted in two different ways. One hypothesis is that it reflects an overwhelming consumption of TGF- β 1 at the time of active neural inflammation. Whereas active TGF- β 1, which is rapidly taken up and degraded by tissues, has a very short half life, latent inactive TGF- β 1 bound to α 2-macroglobulin is largely confined to the circulation, and has a greatly extended half life.³⁸ At the time of activation of latent TGF- β 1 by proteases at cell surfaces, α 2-macroglobulin undergoes internalisation and degradation.³⁸ In the case of pronounced consumption of TGF- β 1, a concurrent decrease of circulating α 2-macroglobulin could be found. We have previously shown that this is not the case in patients with CIDP and POEMS syndrome, who have greatly decreased concentrations of TGF- β 1 plasma, contrasting with normal circulating α 2-macroglobulin concentrations.¹⁶ Another hypothesis is that down regulation of TGF- β 1 production occurs in the progression phase of Guillain-Barré syndrome. This hypothesis is in keeping with the previous report of a decrease in TGF- β 1 mRNA concentrations in circulating mononuclear cells at the time of relapses of multiple sclerosis.¹⁵ In this hypothesis it seems likely that downregulation of TGF- β 1 participates in the inflammatory process of Guillain-Barré syndrome. Experiments in TGF- β 1 null mice have shown that suppressed TGF- β 1 production is associated with severe multifocal tissue inflammation.^{17, 18} In the same way, inhibition of TGF- β 1 activity by local administration of relevant antibodies was shown to increase tissue inflammation in a model of muscle regeneration.³⁹

In EAN, a peak of mRNA TGF- β 1 expression is found in nerve at the onset of recovery.^{13 14 40} Upregulation of TGF- β 1 at the time of recovery from Guillain-Barré syndrome is consistent with the role ascribed to TGF- β 1 in termination of the inflammatory response and in tissue repair.^{13 41} TGF- β 1 also induces Schwann cell proliferation in neuron free cultures,^{42 43} regulates Schwann cell differentiation,⁴³ and behaves as a neurotrophic factor.^{44 45}

We conclude that (1) TGF- β 1 is down regulated in the early phase of Guillain-Barré syndrome; (2) plasma concentrations of TGF- β 1 increase with motor function until early recovery; (3) TGF- β 1 is a key cytokine in the homeostasis of the inflammatory reaction in Guillain-Barré syndrome, and, unlike TNF- α , is stable and normally present in the circulation. We think that TGF- β 1 should be evaluated as a marker of the inflammatory reaction in patients with Guillain-Barré syndrome.

This study has been supported by a Projet Hospitalier de Recherche Clinique (AP/HP). We thank Mrs Piedouillet and Mrs Poron for excellent technical assistance, and Mrs Castet, Mrs Morge, and the nurses of the Department of Neurology, Henri Mondor Hospital for collecting blood samples. We are grateful to Dr Hany Soliman (Laboratoire de Biologie Hormonale, Hôpital Saint-Louis, Paris) for cortisol measurements, and Dr Intrator for measurements of anti-GM1 antibodies

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