The Turkish Journal of Pediatrics 1998; 40: 357-363

PRO – INFLAMMATORY CYTOKINES, LYMPHOCYTE SUBSETS AND INTRAVENOUS IMMUNOGLOBULIN THERAPY IN GUILLAIN – BARRÉ SYNDROME*

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SUMMARY: Tekgül H, Kütükçüler N, Çağlayan S, Tütüncüoğlu S. (Department of Pediatrics, Ege University Faculty of Medicine, İzmir, Turkey). Pro-inflammatory cytokines, lymphocyte subsets and intravenous immunoglobulin therapy in Guillain-Barré syndrome. Turk J Pediatr 1998; 40: 357-363.

Both cell-mediated immunity and humoral factors are involved in the pathogenesis of Guillain-Barré syndrome (GBS). Intravenous immunoglobulin (IVIG) has been reported to be a practical, effective and safe treatment in childhood GBS, although the mode of action of IVIG remains uncertain. We studied pro-inflammatory cytokines (interleukin-2, interleukin-1α and tumor necrosis factor-α) in plasma and cerebrospinal fluid (CSF) and lymphocyte subsets in peripheral blood both in the acute phase and in the recovery period in six children with GBS treated with IVIG. Flow cytometry was used to determine the subsets of lymphocytes in peripheral blood, and cytokines analyses were performed by using ELISA technique. Results were compared with a control group of 20 healthy children. A standard protocol of IVIG (400 mg/kg/day for 5 days) was administered to all the patients. Plasma interleukin-2 concentrations and the number of HLA DR+ active T cells in peripheral blood were significantly higher in the acute phase of the disease than in the recovery period and in healthy controls. There was no significant difference in the other cytokine concentrations in plasma and CSF or in the other lymphocyte subsets in peripheral blood. Our data indicate that IVIG may provide its possible theurapeutic effect by acting in the cell-mediated immunity in GBS patients. *Key words: Guillain-Barré syndrome, cellular immunity, lymphocyte subsets, cytokines, intravenous immunoglobulin.*

Guillain-Barré syndrome (GBS), a demyelinating disease of peripheral nerves, results from abberant immune responses directed against components of the peripheral nerve[1]. Both cell-mediated immunity and humoral factor are involved in the pathogenesis of the disease[1-5]. The close histological resemblance between experimental allergic neuritis and GBS suggests a common autoimmune pathogenesis[2]. The therapeutic efficacy of plasmapheresis treatment and intravenous immunoglobulin (IVIG) and the presence of circulating antibodies directed to the peripheral nerves also evidence an immune-mediated disorder of peripheral nerves[6-8]. The release of pro-inflammatory cytokines such as interleukin-1α (IL-1α) and tumor necrosis factor-α (TNF-α) from macrophages may have a myelinotoxic effect in the peripheral nerves[3-5].

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In this study, we the plasma and cerebrospinal fluid (CSF) concentrations of cytokines (interleukin-2, IL-1α and TNF-α) and lymphocyte subsets in peripheral blood of patients with GBS both in the acute phase and in the recovery period of the disease. The objectives of the study were (1) to investigate the role of cell-mediated immunity in the pathogenesis of GBS, (2) and to determine the effect of IVIG treatment on the cell-mediated immunity and pro-inflammatory cytokines.

Material and Methods

Six children with GBS diagnosed by using NINCDS'78 criteria[9] were included in the study. Their ages were between 2.5-13 years, (8.2 ± 3.8) and four of them were girls. Four patients had a history of acute respiratory infectilon; two had no history of any acute infection. The clinical manifestations were motor weakness (6/6), cranial nerve involvement (1/6) and disturbance of sensation (1/6). One of the patients with maximal motor deficit required ventilation assistance. All the patients showed albuminocytologic dissociation features and a reduction in nerve conduction velocity.

Intravenous immunoglobulin (Sandoglobulin, Sandoz Pharmaceuticals, 400 mg/kg/day for five days) was administered to all the cases. The patients started recovering within four days and the mean time to recovery of independent walking was 14 days. Three were followed for a 12-month period in the outpatient clinic and no relapse was observed. Peripheral blood and CSF were taken before the administration of IVIG (acute phase) and also when the patients reached unaided walking (recovery period).

Twenty healthy children (10 girls, 10 boys) with an age range of two to 17 years (mean 12.4 ± 8.4) were included as a control group for peripheral blood lymphocyte subsets and plasma cytokine concentrations.

Enumeration of peripheral blood mononuclear cell subsets: the percentage of total T (CD3+ cells) and B (CD19+ cells) lymphocytes, T helper/inducer (CD4+ cells) and T suppressor/cytotoxic (CD8+ cells) lymphocytes, natural killer cells (CD16+56+ cells) and HLA-DR+ active T cells were analyzed with monoclonal antibodies labeled with either fluorescein isothiocyanate (FITC) or phycoerthrin (PE) (Becton-Dickinson, Belgium) and with flow cytometry (FACSCAN, Becton-Dickinson). Briefly, peripheral blood cells and each monoclonal antibody were incubated for 30 min at room temperature in the dark and red blood cells were then lysed with FACS lysing solution (Becton-Dickinson). The cells were then washed with phosphate-buffered solution and were analyzed by flow cytometry. Absolute numbers of lymphocyte subsets were calculated on the basis of total and differential white cell counts and the percentage of total T lymphocytes with T cell markers.

Plasma and CSF cytokine levels: IL-2, IL-1 alpha, and TNF-alpha (Amersham, Aylesbury, UK) concentrations were measured using enzyme-linked immunosorbent assay (ELISA). The minimum detectable concentratilons of IL-2, IL-1α and TNF-α

by this method are 0.6 pg/ml, 0.1 pg/ml and 0.1 pg/ml, respectively. The assays have no cross-reactivity with the other cytokines. Inter/intra-assay variabilities were less than 10 percent. The samples were stored at -30 °C until use.

Results of lymphocyte phenotyping and cytokine concentrations were compared using Mann-Whitney U test.

Results

The percentage and absolute counts of lymphocyte subsets in peripheral blood of patients with GBS and in healthy controls are listed in Table I. No significant difference was found in lymphocyte subsets between patient and control groups except HLA-DR+ active T cells. In the acute phase of GBS, the percentages (11.2 ± 3.0%) and absolute counts (540 ± 200/mm^3) of active T cells were highly elevated when compared with the data obtained during the recovery period (5.3 ± 1.5%, 220 ± 60/mm^3) and in healthy children (4.5 ± 2.4%, 190 ± 80/mm^3) ($p < 0.05$). On the other hand, no significant difference was observed in active T cells in the recovery period and in healthy children ($p > 0.05$). Plasma IL-2 concentrations were found to be significantly higher in the acute phase (3.32 ± 1.74 pg/ml) when compared with the recovery period (0.90 ± 0.62 pg/ml) and healthy controls (non-detectable) ($p < 0.05$) (Table II). In contrast, plasma IL-1α and TNF-α levels in the acute phase and recovery period and in healthy controls were similar ($p > 0.05$) (Table II).

Table I: Percentage and Absolute Counts of Lymphocyte Subsets in Patients with GBS and in Healthy Controls

		I. Acute Phase n = 6	II. Recovery Phase n = 6	III. Control n = 20
Total lymphocyte	(ab)	4.44 ± 0.97	3.90 ± 0.76	4.60 ± 1.27
CD3+	(%)	57.2 ± 8.00	59.0 ± 14.5	73.0 ± 6.50
	(ab)	2.92 ± 0.56	2.58 ± 0.72	3.56 ± 0.76
CD19+	(%)	23.4 ± 5.30	15.0 ± 4.30	14.0 ± 4.20
	(ab)	1.08 ± 0.42	0.80 ± 0.30	0.72 ± 0.28
CD4+	(%)	34.6 ± 10.5	35.0 ± 3.00	44.0 ± 7.60
	(ab)	1.80 ± 0.52	1.42 ± 0.22	2.08 ± 0.43
CD8+	(%)	33.2 ± 11.0	30.0 ± 13.1	33.0 ± 5.40
	(ab)	1.58 ± 0.48	1.26 ± 0.66	1.66 ± 0.32
CD4/8		1.22 ± 0.61	1.30 ± 0.60	1.40 ± 0.20
CD16+56+	(%)	16.4 ± 10.5	23.3 ± 13.6	14.1 ± 4.20
	(ab)	0.72 ± 0.52	0.96 ± 0.64	0.84 ± 0.16
HLA-DR+T cell	(%)	11.2 ± 3.00*	5.30 ± 1.50	4.50 ± 2.40
(active T cell)	(ab)	0.54 ± 0.20*	0.22 ± 0.06	0.19 ± 0.08

ab: absolute count. 103 cells/mm3. * I-II: $p < 0.05$, I-III: $p < 0.05$, II-III: $p > 0.05$.

Table II: Plasma IL-2, IL1α and TNF-α Concentrations in Patients with GBS and in Healthy Controls

	I. Acute Phase n = 6	II. Recovery Period n = 6	III. Controls n = 20
IL-2 (pg/ml)	3.32 ± 1.74*	0.99 ± 0.62	ND
IL-1α (pg/ml)	1.08 ± 0.21	1.14 ± 0.45	2.88 ± 1.67
TNF-α	0.23 ± 0.48	0.20 ± 0.34	2.13 ± 2.28

*I-II. $p < 0.05$, I-III: $p < 0.05$, ND: non-detectable concentrations.

CSF IL-2 concentrations were non-detectable in all the samples, both at the onset of the disease and in the recovery period (Table III). There was no significant elevation between CSF IL-1α and TNF-α levels during the acute and recovery periods of the disease ($p > 0.05$, Table III).

Table III: CSF IL-2, IL-1α and TNF-α Concentrations of Patients with GBS ($p > 0.05$)

	I Acute Phase	II. Recovery Period
IL-2 (pg/ml)	ND	ND
IL-1a (pg/ml)	0.88 ± 0.65	0.93 ± 1.17
TNF-a (pg/ml)	0.42 ± 0.83	0.4 ± 0.7

ND: non-detectable concentrations.

Discussion

In GBS, evidence has indicated probable derangement of T cell subsets and alterations of T lymphocyte functions[10, 11]. However, the neuritogen or pathogen that triggers GBS is still not known. One immunocytochemical study about infiltrating lymphocytes on peripheral nerves revealed a predominance of CD4+ T helper/inducer cells over CD8+ suppressor/cytotoxic T cells and the presence of some B cells[12]. In Winer et al.'s[2] and Iqbal et al.'s[10] studies, CD8+ lymphocytes in peripheral blood were found to be significantly reduced, but total lymphocytes, total T and CD4+ cells were not altered. In contrast to these results, in our study CD8+ cells in peripheral blood of patients with GBS were not altered in the acute phase, nor were CD3+, CD4+, CD19+, CD16+56+ cells (Table I).

In GBS patients, circulating T cells that carry on their surface activation markers HLA-DR and interleukin-2 receptor (IL-2R) were increased in number[11, 13]. Increased plasma concentrations of soluble IL-2R (sIL-2R) indicate the presence of circulating activated T cells. In Hartung et al.'s study[11], higher concentrations of sIL-2R shed from activated T cells were measured in the plasma of patients with GBS and correlated with disease activity. On the other hand, circulating IL-2 concentration has been found in increased amounts in GBS and declined in parallel with clinical recovery[14]. In our study, in the acute phase of GBS, both

an increased percentage of HLA-DR+ active T cells in peripheral blood and increased levels of plasma IL-2 in the acute phase strongly indicate the activation of the cellular immune system. As the patients recovered, active T cells and plasma IL-2 levels declined to normal levels. As was expected, the CSF IL-2 concentrations were non-detectable in all the samples both at the onset of the disease and in the recovery period because lymphocytes do not infiltrate CSF in GBS, but also because IL-2 probably does not tranasfer from peripheral blood to CSF.

In GBS, the mechanisms by which circulating active T cells come to the peripheral nervous system are unknown. However, activated T cells could be relevant to the pathogenesis in several ways. First, activated CD4+ cells of the Th2 phenotype synthesize IL-4, IL-5, IL-6 and may cause B cell proliferation and transformation into plasma cells which produce antibodies against peripheral myelin components[1]. Second, the Th1 phenotype, which synthesizes IFN-γ, IL-2, TNF-α and β, could damage myelin by secreting pro-inflammatory and myelinotoxic cytokines and could operate by recruiting macrophages to exert nerve damage[1]. Macrophages have been identified as major cellular components in the tissue lesion, and their decisive role in mediating autoimmune peripheral nerve injury has been proven in the animal model of EAN[11]. It was shown that macrophages act as antigen presenters and are of pivotal importance in the amplification of immune-mediated demyelination by mechanisms including phagocytosis and the release of pro-inflammatory cytokines[3]. On the basis of these observations, we investigated cytokine production (IL-1α, TNF-α) of macrophages in plasma in order to determine their contribution in the pathogenesis of GBS. Both the plasma levels of TNF-α and IL-1α were not found to be elevated and were at low levels as was observed in healthy children. In Sharief et al.'s study[15], ony 20-50 percent of GBS patients have raised serum levels of TNF-α. The low levels of these pro-inflammatory cytokines in plasma suggested that the production of these cytokines by peripheral blood monocytes and macrophages was inhibited or not effected throughout the course of GBS. The serum levels of inhibitors of these cytokines, such as interleukin-1 receptor antagonist (IL-1ra) and soluble TNF-receptors (TNF-sR), have to be measured because in many autoimmune diseases these inhibitors were found to be elevated furthermore, they are important regulatory components of IL-1 and TNF mediated inflammation and immune response[16].

We also measured CSF concentration of IL-1α and TNF-α and tried to determine if there was a passive transfer or overproduction of these cytokines. Similar to results seen in the plasma, they were not found to be elevated in CSF in the acute period of the disease (Table III). Cellular infiltration including monocytes and macrophages of CSF in GBS is minimal and we did not expect to find elevated cytokine levels. On the other hand, it is not possible to suggest the presence or absence of a passive transfer of IL-1α and TNF-α into CSF because of the very low levels on both sides.

The decline to normal levels for active T cells and plasma IL-2 concentrations may be due to IVIG treatment or it may be just laboratory evidence of a recovery period that can be observed following any type of immunotherapy. However, it would have been important to support this claim by studying another group of patients with GBS but without IVIG. Furthermore, the mode of action of IVIG remains uncertain, although several mechanisms have been proposed. It has been reported that IVIG contains anti-idiotype antibodies against auto-antibodies and also down regulates cytokine production (IL-2, TNF-α and β). In addition, it has an interference effect with superantigen-induced T cell activation by antitoxin antibodies[17].

IVIG therapy shortened the duration of disability in GBS in comparison with standard supportive treatment in a large, open, controlled trial[18, 19]. Although IVIG is potentially a safer treatment, relapse or disease progression after treatment has been reported[18]. In our patients, IVIG shortened the average duration of the disease with no relapse after 12 months of follow up.

In conclusion, elevation of IL2 concentrations and an increased number of activated T-cells in the acute phase and a decline to normal levels in the recovery phase indicate that IVIG may play a role in the suppression of cell-mediated immunity in patients with GBS.

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