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IMMUNOLOGICAL OBSERVATIONS IN GUILLAIN-BARRÉ SYNDROME

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The results of some immunological analyses in 12 patients with the acute Guillain-Barré syndrome (GBS) are presented. The mean cerebrospinal fluid (CSF) concentrations of IgG and IgA were significantly increased. A longitudinal study of CSF IgG concentrations in 5 patients shows that the recovery period of the disease is not regularly accompanied by a decline of the IgG level. The finding of significantly reduced percentages of active T cells in the peripheral blood of patients with the acute GBS in comparison with the controls supports the view of the cellular immunity role in the pathogenesis of the disease. With the exception of HLA—B_5 and HLA—B_{15}, no other individual antigen was significantly more present in either the cases or controls.

Immune-mediated mechanisms are assumed to contribute to the pathogenesis of the Guillain-Barré syndrome[1–3]. Numerous laboratory analyses have shown a strikingly disturbed immunity in the Guillain-Barré syndrome, but none of the observed changes either in humoral[4–6] or in cellular[3,7] immunity is characteristic of the disease. Some conflicting results in these analyses may be explained by the heterogeneity of Guillain-Barré syndrome patient group, i.e. the analyses were not made at the same stages during the course of the disease[5].

In this study we analysed a relatively homogeneous group of 12 Guillain-Barré syndrome patients with a moderate form of the disease and the duration shorter than three months with a good outcome in the majority of cases. We attempted to establish: a) an initial, pre-treatment pattern of immunologic response, and b) the CSF IgG pattern spanning the acute and recovery phases.

MATERIALS AND METHODS

Patients

The sample of population consisted of 12 patients, 10 males and 2 females, with a clinically definite Guillain-Barré syndrome (8; Table 1).

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Table 1.

*Diagnostic criteria for Guillain-Barré syndrome**

Required criteria	Supportive criteria
Progressive weakness of more than one limb	*Clinical*
Areflexia	Relatively symmetrical weakness
Duration of progression less than 4 weeks	Relatively mild sensory signs
	Cranial nerve involvement, especially facial nerve
	Autonomic dysfunction; vasomotor instability
	Absence of fever with neuropathic symptoms
	CSF (after the first week)
	CSF protein elevated
	CSF cell count more or less normal
	Neurophysiological
	Slowing of nerve conduction suggestive of demyelination

* Modif cation of NINCDS Committee Criteria (see Leibowitz et Hughes, 1983)

Their average age was 40.7 years, ranging from 14 to 73. None of the patients had anamnestically any antecendent infection or immunization procedure. The first complaints were usually paresthesia and/or motor weakness followed by progression from distal to proximal parts in the majority of cases and with a maximum, i.e. paralysis and absent tendon reflexes, toward the end of the first month of illness. The patients who developed respiratory failure and required ventilation were excluded from the study. Most of them recovered completely and one quarter showed some residual signs (motor weakness and/or abolished tendon reflexes). All patients were receiving steroids after the initial samples of their blood and CSF had been taken. The effect of treatment was not assessed in our material because no systematic comparative studies with different treatments were made. The control population consisted of patients with other noninflammatory neurologic diseases (headache, ischialgia, neurosis) and the corresponding number of age-and sex-matched healthy persons in some analyses. The sizes of some of the control and analytical groups were different because during the period of investigation (1979—1987) not all of the parameters were estimated simultaneously, thus, on the one side, only simultaneous samples of the CSF and blood were taken in order to study the CSF IgG and IgA concentrations, whereas, on the other, the number of healthy persons was complete by the time of the last corresponding analysis.

CSF and serum studies

The CSF total protein concentration was determined by the Lowry method[9]. Concentrations of immunoglobulins and C_3 component of the complement were determined by the radial immunodiffusion methods[10]. Lymphocyte subpopulations in the peripheral blood were identified by

the rosette forming method somewhat modified in our laboratory[11]. Typing for HLA antigens was performed by the routine microlymphocytotoxicity test[12].

Statistical analysis

The statistical methods utilized were Student's t-test and the relative risk (RR) calculation for HLA-frequencies.

RESULTS

Immunoglobulins in serum

Our results show that there were no statistically significant deviations in IgG, IgA and IgM levels between the GBS patients and the controls (Table 2).

Table 2.

Immunoglobulins and C_3 concentrations (mean g/L±SD) in serum from patients with acute Guillain-Barré syndrome and healthy controls

Group	IgM	IgA	IgG	C_3
GBS (n=12)	2.36 ± 1.21	3.01 ± 1.26	15.43 ± 4.67	1.59 ± 0.47
Controls (n=20)	1.96 ± 0.79	2.60 ± 0.74	13.76 ± 2.71	1.12 ± 0.20
Levels of significance (p)*	NS	NS	NS	<0.01

* Student's t-test; NS = not significant; GBS — patients with Guillain-Barré syndrome

Immunoglobulins in CSF

The mean CSF protein concentration in 8 patients was significantly higher in the GBS patients than in the controls (0.7±0.9 g/L : 0.35±0.2

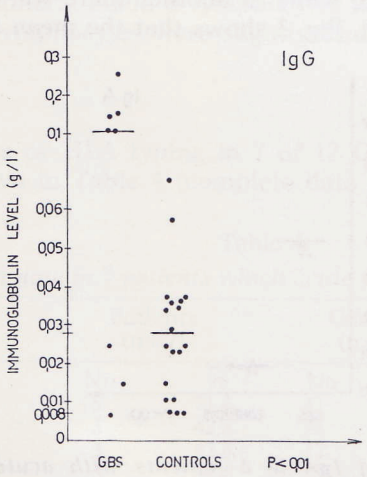

Fig. 1. *CSF concentrations of IgG in 8 patients with acute Guillain-Barré syndrome versus 19 patients with noninflammatory neurologic diseases. Bars indicate median values in each population*

g/L ; $p<0.01$). The mean concentration of IgG in the initial CSF samples of the GBS patients (Fig. 1) was significantly higher when compared with the controls ($p<0.01$). The pattern of changes in CSF IgG concentrations in 5 of 8 Guillain-Barré syndrome patients from this group (analysed during the following few weeks) was different in each patient, although the recovery ensued regularly in all of them (Fig. 2). High initial values

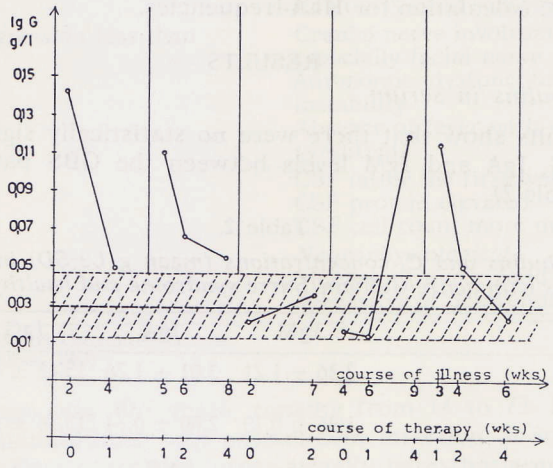

Fig. 2. *Pattern of CSF immunoglobulin G changes in 5 patients with acute Guillain-Barré syndrome obtained during their illness. Dotted line indicates normal mean value*

of IgG concentrations (obtained prior to therapy) decreased in 3 patients to normal after several weeks. However, in one patient with the normal initial level of CSF IgG, a significant increase during the recovery period was observed, while in another there were no deviations from the normal level at all. Fig. 3 shows that the mean and borderline

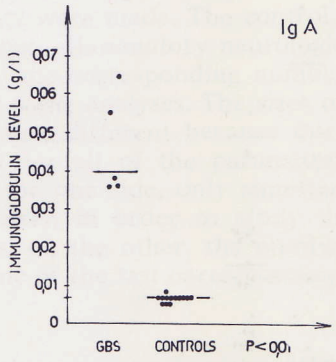

Fig. 3. *CSF concentrations of IgA in 6 patients with acute Guillain-Barré syndrome versus 13 patients with noninflammatory neurologic diseases. Bars indicate median values in each population.*

IgA values in the CSF were significantly higher in all examined Guillain-Barré syndrome patients in comparison with the controls ($p<0.01$).

Complement (C_3) concentration in serum

The serum concentrations of C_3 in the Guillain-Barré syndrome patients were signifiiantly higher in comparison with the controls ($p < 0.01$) (Table 2).

Lymphocyte subpopulation in peripheral blood

Table 3 shows the percentage of total T and active T lymphocytes as well as B lympocytes in the pheripheral blood of the Guillain-Barré syndrome patients and the controls.

Table 3.

Lymphocyte subpopulations in peripheral blood from patients with acute Guillain-Barré syndrome and from normal controls

Group	Active E-RFC %	Total E-RFC %	EAC-RFC %
GBS (n=12)	21.1 ± 11	50.5 ± 3.7	18.2 ± 5.7
Controls (n=13)	34.5 ± 8.6	57.7 ± 5	22.5 ± 7.1
Levels of significance (p)*	<0.01	NS	NS

* Student's t-test; NS = not significant

There was a statisticaly significant decrease of active T lymphocytes in the GBS patients versus the controls ($p<0.01$). It must be pointed out again that the tests were conducted prior to the therapy. The percentage of peripheral blood B lymphocytes was within the normal range, while total T lymphocytes were slightly decreased when compared with the controls.

HLA typing

The results of HLA typing in 7 of 12 Guillain-Barré syndrome patients are shown in Table 4 (complete data are not presented). HLA-B_6

Table 4.

Some HLA data in 7 patients which acute Guillain-Barré syndrome

Antigen	Patients (n=7)		Controls (n=91)		Relative risk
	No	%	No	%	
A_3	5	71.4	47	42.8	NS
A_9	3	42.8	24	21.9	NS
B_5	3	42.8	12	11	4.9
B_7	3	42.8	23	21	NS
B_{27}	2	28.5	3	2.7	11.7

NS = not significant

and B_{17} were found to be increased in the Guillain-Barré syndrome patients (42.8% vs 11% in the controls for B_5, $p < 0.01$; 28.5% vs 2.7% for B_{17}, $p < 0.01$) with a relative risk of 4.9 and 11.7, respectively.

DISCUSSION

The results of our study have shown that immunoglobulins in the serum from patients with the acute Guillain-Barré syndrome were within the normal range. This agrees with data reported by Link,[12] and Amarenco et al.[5] On the other hand, Dowling et al.[4] and Vedeler et al.[13] showed a significant rise, particularly in the IgM fraction, during the acute phase of the Guillain-Barré syndrome. In addition, Dowling et al.[4] found a serologic evidence of recent infections with cytomegalovirus, Mycoplasma pneumoniae or Epstein-Barr virus, in two thirds of the patients with an elevated level of IgM serum. Following a complement fixation test in all Guillain-Barré syndrome patients and the ELISA test in five of them (for Mycoplasma pneumoniae and viruses), no significant titres were registered in any of our patients (data are not presented). These conflicting results may be in part explained by restricted values of serologic test used for diagnosing recent infections with the DNA core agents mentioned, because most of these infections are indeed of recurrent nature. Newer methods, utilizing monoclonal antibodies or DNA hybridization techniques, may eventually resolve this problem.

We found a significant elevation of the IgA level in the CSF (Fig. 3) in all examined Guillain-Barré syndrome patients (6 of 6; $p<0.01$), whereas the CSF IgG level was significantly elevated in 5 of 8 patients ($p<0.01$; Fig. 1).

Some authors reported that the increased CSF concentration of IgG in patients with the acute Guillain-Barré syndrome may primarily be due to the leakage from the serum through a damaged blood-CSF barrier[14]. Contrary to this, others showed that the increased CSF IgG in the acute (paralytic) phase of the Guillain-Barré syndrome is primarily caused by a greater local CNS synthesis of IgG[5]. While the capacity of lymphoid cells in the nervous system to produce IgG and IgA was demonstrated in vitro for more than 20 years ago[15], their activity in vivo can be calculated only by empirically derived formulas[16–18]. One critical review of the clinical usefulness of these formulas showed that none of them is sufficiently valid for the calculation of intrathecally synthesized immunoglobulins[19]. Therefore, our attempt in this work was to estimate only the dynamics of CSF IgG changes during the clinical course of the Guillain-Barré syndrome. This longitudinal study showed that in 3 of 5 Guillain-Barré syndrome patients, who had a high initial CSF IgG concentrations, clinical improvement was associated with an CSF IgG decline to the normal level. In contrast, there were normal CSF IgG concentrations in two of our patients on adminission, followed by a significant increase during the recovery period in one of them.

Obviously, our data cannot support any of the explanations for the increased CSF IgG (or CSF IgA) in the acute Guillain-Barré syndrome, but

they suggest that a positive correlation between CSF IgG concentrations and the severity and/or duration of the disease that could be seen in Guillain-Barré syndrome patients is not the rule. Further studies must be conducted to reach a definite conclusion about these observation.

Median plasma levels of C_3, as shown in Table 2, were significantly elevated when compared with samples from the control group (1.12 versus 1.59; $p<0.01$). Similarly, Tonnenssen et al.[20] found a significant rise in serum C_3 (and serum C_4) in severe Guillain-Barré syndrome cases, while Link[31] found no significant variation of the serum complement level. It must be noted that the number of Guillain-Barré syndrome patients was small in all three reports.

Some recent data indicate that the complement system is activated in the CSF of patients with the acute Guillain-Barré syndrome[3,6,21]. Some of these arguments[5] indicate the presence of local CNS consumption of C_3 and, moreover, some of them suggest that activated terminal complement components may directly participate in the process of demyelination.[21]

Our study of lymphocyte subpopulations in the peripheral blood of patients with the acute Guillain-Barré syndrome showed a significantly lower number of active T lymphocytes when compared with those of controls (Table 3). This result confirmed some earlier observations[22,23]. It is interesting to compare our results obtained in patiens with the acute Guillain-Barré syndrome with the similar results we observed in our patients with active multiple sclerosis[24,25]. In both analyses the picture of a significant reduction of active T lymphocytes during the acute phases of diseases dominates. Taken together, these results support the view that cell-medicated immunity plays an important role in the pathogenesis of demyelination, regardless of whether or not it occurs in the central or in the peripheral nervous system and whether or not the defects observed are primary or secondary events in the tissue damage process.

The results of HLA analysis in our patients showed that the frequency of HLA-B_5 and B_{17} antigens was increased in Guillain-Barrè syndrome patients in comparison with controls, but the relative risk calculation revealed no stronger association of the Guillain-Barré syndrome with a specific HLA antigen. This observation is in accordance with some earlier reports[26,27].

In conclusion, it is clear that longitudinal studies of CSF and peripheral blood lymphocyte subpopulations, immunoglobulins and complement components will be necessary in order to explore the relationship between the observed immunologic alterations and the evolution of the Guillain-Barré syndrome.

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SAŽETAK

IMUNOLOŠKA ANALIZA GUILLAIN-BARRÉOVA SINDROMA

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Prikazani su rezultati nekih imunoloških pretraga izvršenih u 12 bolesnika s akutnim Guillain-Barréovim sindromom (dijagnostički kriteriji prema Asburyju i sur. 1978). Analizirana je koncentracija imunoglobulina u serumu i cerebrospinalnom likvoru (CSL), koncentracija C_3 komponente komplementa u serumu, limfocitne subpopulacije u krvi, te HLA fenotip bolesnika s Guillain-Barréovim sindromom. Kontrolne grupe sačinjavali su zdravi ispitanici i bolesnici s drugim neinflamatornim neurološkim bolestima, odgovarajuće spolne i dobne razdiobe.

Rezultati analize serumske razine imunoglobulinskih frakcija nisu pokazali odstupanja od normale, ali je razina C_3 komponente komplementa bila statistički značajno veća u skupini bolesnika u odnosu na kontrolnu skupinu ($p<0.01$). Podaci u literaturi o razini C_3 komponente komplementa u bolesnika s Guillain-Barréovim sindromom su različiti, a osim toga je i broj ispitanika u svim tim prikazima bio relativno malen da bi se neki zaključak mogao generalizirati. Srednja koncentracija IgG i IgA u CSL bila je u grupi bolesnika s Guillain-Barréovim sindromom značajno veća u odnosu na kontrolu ($p < 0,01$), što ukazuje bilo na mogućnost povećane intratekalne sinteze imunoglobulina, bilo na mogućnost njihova prelaska kroz oštećenu krvno-moždanu barijeru. Indeks intratekalne sinteze imunoglobulina autori u ovome radu nisu određivali zbog neujednačenosti današnjih kriterija (formula).

Praćenje dinamike koncentracije IgG u CSL u pojedinih bolesnika s akutnim Guillain-Barréovim sindromom pokazalo je da je u većine bolesnika oporavak praćen padom razine IgG na normalne vrijednosti, što međutim nije i pravilo, kako bi se po nekim navodima iz literature moglo zaključiti. Analiza limfocitnih subpopulacija u krvi pokazala je da u akutnom Guillain-Barréovu sindromu postoji značajan pad aktivnih T limfocita u odnosu na kontrolne vrijednosti, što potvrđuje bitnu ulogu stanične imunoreakcije u patogenezi demijelinizacije u perifernom nervnom sustavu. HLA fenotipska analiza pokazala je nešto učestaliju frekvenciju HLA-B_5 i B_{17} antigena u bolesnika s Guillain-Barréovim sindromima u odnosu na kontrolnu populaciju, ali izračunavanje relativnog rizika nije pokazalo statistički značajniju povezanost tih entiteta.