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IMMUNOLOGICAL MARKERS IN PATIENTS WITH DIFFERENT FORMS OF VIRAL HEPATITIS B TREATED BY "CONVENTIONAL" THERAPY OR WITH HuIFN ALPHA

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Summary. — Selected immunological, biochemical, and other viral hepatitis B (VH-B) markers were followed and analysed during "conventional" or human interferon alpha (HuIFN alpha) therapy of patients with different forms of VH-B. The immunological data obtained from "conventionally"-treated acute hepatitis B (AH-B), prolonged acute hepatitis B (AH-BP) or chronic active hepatitis B (CAH-B) patients disclosed differences unsatisfactory for comparison of the influence of HuIFN alpha therapy on changes of the immunological markers. More valuable data were obtained through continuous registration of the dynamics of selected blood markers. Partial effects on immunological parameters were seen after HuIFN alpha administration to 2 patients with developing CAH-B infection. Progression of the disease was markedly halted in these both patients after IFN treatment.

Key words: *viral hepatitis* B; *interferon therapy*; *immunological markers*

Introduction

During last years a substantial amount of information was collected concerning therapeutical application of HuIFN alpha in chronic viral hepatitis B (Gregory *et al.*, 1984, 1986; Carreno *et al.*, 1987; Stanček *et al.*, 1988, 1989). In the majority of trials IFN alone or in combination with chemotherapy or immunomodulating approaches exhibited partial therapeutic effects. Little is known about immunological events which might be affected by exogenous IFN administration to CAH-B patients. Immunopathogenesis of chronic VH-B infection is not satisfactorily understood either. There are reports pointing on the importance of cellular defense mechanisms and their relation to IFN for recovery from VH-B infection and its chronic development

(Vladutin, 1978; Carter *et al.*, 1979; Kakumu *et al.*, 1988). Considerable attention was focussed on lymphokine-activated killer (LAK) or natural killer (NK) cells involvement in the pathogenesis and recovery from various viral and malignant diseases (Herberman, 1984; Farrar *et al.*, 1984; Kimoto and Taguchi, 1987; Cauda *et al.*, 1988).

In our previous reports we concentrated mostly on the aetiological markers and their changes during "conventional" therapy, during administration of HuIFN alpha alone or in combined therapy of prolonged or chronic forms of VH-B infection (Stanček *et al.*, 1987, 1988, 1989). The present report brings some selected immunological data obtained through monitoring of patients with different forms of VH-B treated either by "conventional" or HuIFN alpha therapy.

Materials and Methods

Immunological markers detection and normal values. Detection of immunological blood markers was done by commonly used technics mostly based on commercially available kits (SEVAC, Prague; IMUNA, Šarišské Michaľany). Normal values: T lymphocytes (T ly): 55%–65%; B lymphocytes (B ly): 15–25%; phytohaemaglutinine-T ly – transformation test (LTT-PHA): 80%–120%; immunoregulation index (IRI e.i. OKT 4 : OKT 8 ratio); phagocytic activity (PA): 30%–70%; phagocytic index (PI): 2.52 ± 0.4; C3 complement (C3): 0.80–1.20; C4 complement (C4): 0.18–0.42 g/l; circulating immune complexes (CIC): up to 45; IgA: 1.0–5.0 g/l; IgG: 7.5–16 g/l; IgM: 0.45–2.95 g/l (all classes of immunoglobulins were evaluated further within the given ranges according to age and sex of the patients).

Aetiological markers detection. All hepatitis B virus (HBV) markers were checked in patients' sera using commercially available ELISA or RIA tests (ABBOTT, SORIN, SEVAC) according to manufacturers recommendations (see also Stanček *et al.*, 1987).

Biochemical markers detection and normal values. Most of the biochemical markers were estimated by commercially available tests. Normal values: alanine-aminotransferase (ALT): <0.35 microcatals/ml; bilirubin-total (Bi-t): <25 micromol/l.

HuIFN alpha (natural or recombinant) therapy: 3×10^6 I.U. a week given i.m. or in gradually increasing doses from 5×10^4 to 10^6 I.U. i.m. daily for 2 weeks, then 10^6 I.U. i.m. 2–3 times a week for several weeks or months. The total IFN dose was up to 15×10^7 I.U. given either continuously for a period of 4 to 28 weeks or in once-twice repeated 4–12 weeks courses.

Results

Different forms of VH-B infection: statistical evaluation of selected immunological markers in patients under "conventional" therapy

To be able to judge more objectively the influence of HuIFN alpha administration of HB-V infection, commonly checked immunological data were collected from the groups of 34–41 patients with different forms of HB-V infection. The serum samples were taken at the patients' admission to hospital and again at their release after 4-16 weeks of "conventional" treatment (i.e. symptomatic non-specific therapy). The calculated mean values and standard deviations of the checked immunological markers are shown in Table 1. Statistical evaluations of these data showed significant differences between the first and the last serum samples only for PI values variations ($p < 0.01$) of the samples taken from the patients with AH-BP (i.e. AH-B lasting for more than 12 weeks). Most frequent were the significant differences

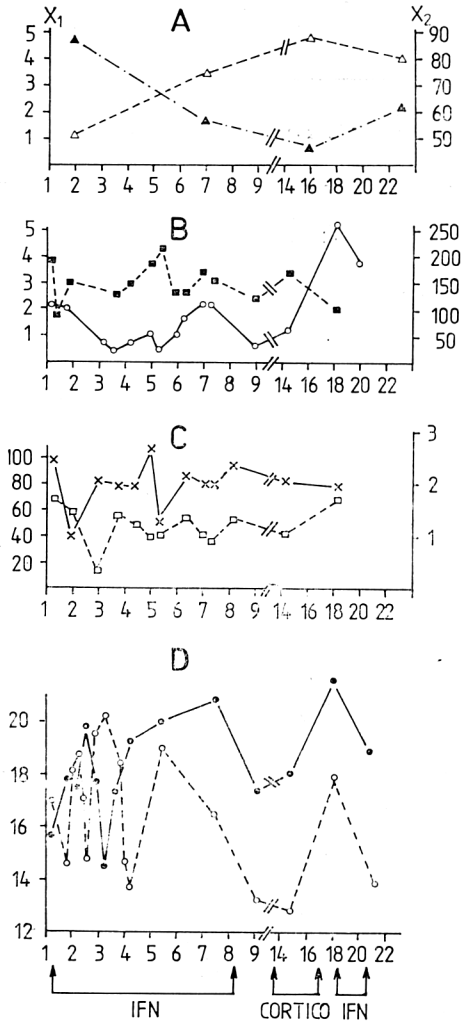

Fig. 1

Dynamics of selected immunological and aetiological markers during HuIFN alpha or corticoid therapies of prolonged AH-B with transition to CAH-B

Fig. 1 A:
X 1 — IRI values (OKT 4 : OKT 8 ratio) ▲.—.—▲
X 2 — LTT — PHA values (%) △ — — △

Fig. 1 B:
X 1 — IgM levels (g/l) ■ — — ■
X 2 — CIC levels (relative values) ○ — ○

Fig. 1 C:
X 1 — PA values (%) □ — — □
X 2 — PI values (relative) × —— ×

Fig. 1 D:
X 1 — HBsAg activity × 10^2 c.p.m. ●—— ●
HBeAg activity × 10^2 c.p.m. ○ — — ○
Abscissa: Time after the first admission to hospital in months.
Arrows indicate the start and the end of either IFN or corticoid therapy.

registered for the mean ($p < 0.05 - < 0.001$) and variation values ($p < 0.01$) of CIC in all 3 types of VH-B infections with the exception of CIC values variations in the samples from CAH-B patients which was not significant. Table 2 shows statistical evaluations of the differences in the mean and variations values between the three groups of patients in the first and the second serum samples. Given are only the data of the selected immunological markers which were significantly different at least in one calculated value of the three compaired groups.

Table 1. Mean values and standard deviations of the selected immunologieal markers in patniets with different forms of VH-B before and after „conventional" therapy

Period of checking	Marker	Clinical for markers of the disease AH-B $\bar{x}$	s. d.	AH-BP $\bar{x}$	s. d.	CAH-B $\bar{x}$	s. d.
At the admition to hospital	T ly	58.87	± 9.49	58.05	± 8.29	58.81	±14.34
	B ly	15.74	± 6.31	13.80	± 4.45	16.44	± 6.03
	PA	54.72	±13.01	59.60	±17.78	51.95	±17.33
	PI	2.08	± 0.28	2.10	± 0.35	1.98	± 0.35
	IgA	4.19	± 1.77	4.10	± 1.44	5.07	± 1.92
	IgG	15.17	± 3.81	14.88	± 3.57	15.07	± 3.62
	IgM	1.86	± 1.55	1.83	± 1.62	1.88	± 1.04
	CIC	71.32	±33.85	66.51	±36.68	69.09	±40.30
At the release from hospital	T ly	60.73	± 8.64	59.61	± 7.31	59.54	± 6.74
	B ly	15.41	± 6.08	15.60	± 6.16	15.59	± 4.65
	PA	57.53	±15.11	62.32	±11.01	55.58	±11.72
	PI	2.04	± 0.27	2.04	± 0.23	2.03	± 0.38
	IgA	4.10	± 2.18	3.89	± 2.06	4.76	± 1.77
	IgG	15.17	± 3.95	14.59	± 5.06	14.81	± 3.31
	IgM	1.79	± 1.42	1.94	± 1.37	1.83	± 0.90
	CIC	45.45	±18.65	47.17	±16.82	55.36	±26.36

$\bar{x}$ = mean values
s. d. = standard deviations
n = 34—41 patients in the particular groud

Different forms of VH-B infection: dynamics of selected immunological markers changes during "conventional" therapy

Since the data described above did not show the dynamic of individual marker changes during the course of VH-B infections, we divided obtained values into three groups according to the changes of the dynamics of the checked parameters: increased, decreased or without any significant change. Tables 3 and 4 show accumulated data (total number and %) and "coefficient" i. e. number of increased plus number of decreased values divided by the number of values without a change which reflects the dynamic of immunity marker changes between the first and the second samples. Most frequent was the high coefficient of humoral immunity markers change in AH-BP patients, the lowest coefficient value being obtained from AH-B patients apparently because of the short time interval between the two checked samples. As far as the cellular immunity marker coefficient values is concerned (Table 4) the most frequent changes were seen in CAH-B patients, while the least frequent occurred in AH-B patients. Among individual data the B ly change was less prominent in AH-BP and AH-B patients, PI values changes were most prominent in CAH-B patients (summarized in Table 5). It shows that the prevailing tendency of increasing all chosen cellular immun-

Table 2. Comparison of mean values and standard deviations of the immunologieal parameters between different forms of VH-B infection at the patients admission to and their release from hospital

Period of checking	Marker*	Compared forms of AH-B infection: AH-B/AH-BP U	AH-B/AH-BP F	AH-B/CAH-B U	AH-B/CAH-B F	AH-BP/CAH-B U	AH-BP/CAH-B F
At the admition to hospital	B ly	$p < 0.04$	$p < 0.05$	n.s.	n.s.	n.t.	n.t.
	IgA	n.s.	n.s.	$p < 0.02$	n.s.	n.t.	n.t.
	IgM	n.s.	n.s.	n.s.	$p < 0.01$	n.s.	n.s.
At the release from hospital	PA	$p < 0.04$	n.s.	n.s.	n.s.	$p < 0.007$	n.s.
	PI	n.s.	n.s.	n.s.	$p < 0.06$	n.s.	$p < 0.01$
	IgA	n.s.	n.s.	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
	CIC	n.s.	n.s.	$p < 0.05$	$p < 0.05$	n.s.	$p < 0.01$

* The table shows only markers found signif. different at least once
n = 34—41 patients in the particular group
U = comparison of mean values; F = comparison of standard deviations
n.s. = not significant n.t. = not tested

Table 3. Dynamics of changes of selected humoral immunity markers during hospitalisation of the patients „conventionally"-treated for different forms of VH-B infection

Marker	Clinical form of VH-B	Dymanics of markers changes betwen the initial and final checking: Increased	Decreased	No change	Coefficient*
IgA	AH-B	13**(33.33)***	22(56.41)	4(10.26)	8.75
	AH-BP	9(23.08)	28(71.79)	2(5.13)	18.49
	CAH-B	10(27.78)	25(69.44)	1(2.78)	34.97
IgG	AH-B	17(43.59)	21(53.85)	1(2.56)	38.05
	AH-BP	15(38.46)	23(58.98)	1(2.56)	38.06
	CAH-B	16(44.44)	19(52.78)	1(2.78)	34.97
IgM	AH-B	14(35.90)	19(48.72)	6(15.38)	5.50
	AH-BP	22(56.41)	16(41.03)	1(2.56)	38.06
	CAH-B	16(44.44)	19(52.78)	1(2.78)	34.97
CIC	AH-B	8(21.05)	28(73.69)	2(5.26)	18.01
	AH-BP	12(30.77)	26(66.67)	1(2.56)	38.06
	CAH-B	12(33.33)	22(61.11)	2(5.56)	16.99

* — $\frac{\text{No. of increased + decreased}}{\text{No. without change}}$
** — number of tested sera
*** — percent
n = 35—39 patiens in the particular group

Table 4. Dynamics of changes of selected cellular immunity markers during hospitalisation of patients „conventionally" treated for different forms of VH-B infection

Marker	Clinical form of VH-B	Dynamics of markers changes between the initial and final checking: Increased	Decresed	No change	Coefficient*
	AH-B	20**(51.29)***	11(28.21)	8(20.51)	3.88
T ly	AH-BP	19(46.34)	16(39.03)	6(14.63)	5.84
	CAH-B	18(50.00)	13(36.11)	5(13.89)	6.20
	AH-B	14(35.90)	12(30.77)	13(33.33)	2.00
B ly	AH-BP	18(43.90)	9(21.95)	14(34.15)	1.93
	CAH-B	18(50.00)	16(44.44)	2(5.56)	16.88
	AH-B	21(58.33)	13(36.11)	2(5.56)	16.88
PA	AH-BP	23(56.10)	11(26.83)	7(17.07)	4.86
	CAH-B	20(58.82)	10(29.41)	4(11.77)	7.50
	AH-B	13(36.11)	16(44.45)	7(19.44)	4.14
PI	AH-PP	17(43.59)	19(48.72)	3(7.69)	12.00
	CAB-B	18(52.94)	15(44.12)	1(2.94)	33.01

* $\frac{\text{No. of increased + decreased}}{\text{No. without change}}$

** — number of tested sera

*** — percent

n = 36—41 in the particular group of patients

ity marker values was seen in CAH-B patients and also for T ly in AH-B patients and for PA in AH-B as well as AH-BP patients. Meanwhile the only prevailing decreasing tendency of cellular immunity markers was found for PI values of AH-B and AH-BP patients. On the other hand almost all chosen humoral serum markers (immunoglobulins andCIC) showed depression tendencies in all three groups of VH-B patients with the only exception of previaling increased IgM serum levels of AH-BP patients.

Serum reactant proteinfs i.e. prealbumin, ceruloplasmin, orosomucoid and alpha-1-antitrypsin have also been tested. In the statistical evaluation of the control healthy group and VH-B patients by T-test, significant differences ($p < 0.001$) were found for ceruloplasmin and orosomucoid, the both being increased in VH-B patients reflecting inflammation and cellular destruction in the patients' liver tissues.

Prolonged forms of VH-B infection: changes of selected immunological, biochemical, and aetiological markers during "conventional" or HuIFN alpha therapy

Based on our experience with aetiological and immunological markers follow-up of "conventionally"-treated VH-B patients, we attempted at

Table 5. The most expressed differences in the dynamics of selected immunity markers in the patients „conventionally" -treated for different forms of VH-B infection

Marker	Prevailing dynamics in the type of VH-B infection: Prevailing increases (>50%)			Prevailing decreases (>45%)		
	AH-B	AH-BP	CAH-B	AH-B	AH-BP	CAH-B
T ly	+	−	+	−	−	−
B ly	−	−	+	−	−	−
PA	+	+	+	−	−	−
PI	−	−	+	+	+	−
IgA	−	−	−	+	+	+
IgG	−	−	−/*N* ±/	+	+	+
IgM	−	−	−/*N* ±/	+	−	+
CIC	−	−	−	+	+	+

comparison of selected namely immunological parameters between the group of 73 VH-B "conventionally"-treated patients and 5 patients treated with either natural or recombinant HuIFN alpha. As shown on condensed, schematical Table 6 no substantial differences among the two groups of patients was found and this approach proved almost useless for evaluation of IFN-treatment efficiency.

Early stages of CAH-B infection: dynamics of selected immunological, aetiological, and biochemical markers during HuIFN alpha corticoid therapies

Frequent monitoring of individual patients for selected immunological, aetiological, and biochemical (not shown) data was organized during natural or recombinant HuIFN alpha administration and successive corticoid therapy. Representative data from one out of three treated patients are shown in Fig. 1. During the initial IFN therapy (1st—15th week) cyclic stimulation and inhibition of HBsAg and in reverse course also HBeAg (i.e. HBsAg elevation was accompanied by HBeAg depression and vice versa) was seen. Increased values were registered for OKT 4 (high IRI) and bilirubin and ALT levels (not shown). On the other hand, PI, PA, IgM, CIC, and LTT-PHA activities were rather decreased. In the following weeks (16th—30th week) a parallel dynamic of HBsAg/HBeAg appeared together with normalisation of PI, PA, IgM, decrease of IRI and elevation of LTT-PHA. After a period of only symptomatic ("conventional") therapy, corticoids were introduced (56th—68th week). Sharp parallel elevations of HBsAg/HBeAg levels could be seen together with PA stimulation, increased levels of CIC and LTT-PHA values, further drop of OKT 4 number (low IRI), bilirubin and ALT levels (not shown). Because of rather sharp HBsAg/HBeAg elevation, HuIFN alpha was readministered (74th—82nd week). This was followed by decreased HBsAg/HBeAg levels as well as CIC and LTT-PHA values and IRI elevation.

Table 6. Changes of immunological or biochemical markers during conventional or HuIFN alpha conventional therapy of VH-B

Immunological, biochemical markers	Conventional therapy[1]			HuIFN alpha therapy[2]		
	Before	During	After	Before	During	After
IgG	N	↓	N	↑	↓	↑/N
IgA	N/↑	↓	N/↑	N/↑	↓	N
IgM	N	N/↑	N/↑	N/↑	↓	↑/N
CIC	↑	↑	↑/N	↑	↓	↑/N
C_3	↓/N	—	↓/N/↑	N/↓	↓	N/↓
C_4	—	—	—	↓/N	↓	N/↓
Phagoc. activity	↓/N	↑	↑	N/↓	↓	↓/N
Phagoc. index	N	↓	N	N	↓	N
B ly	N(↓)	↑	N(↑)	↓	N/↑	N/↑
T ly	N(↓)	↑	N	↓/N	N/↓	N/↓
ALT	↑	↑/N	N/↑	↑	↑/N	N/↑
Bilirubin (tot.)	↑	↑/N	N/↑	N/↑	↓/↑	N

N = normal ↑ = increased ↓ = decreased
1 — n = 73 2 — n = 5 — = not tested

Though the above described effects were presumably due mostly to IFN and corticoids administration, no final cure was achieved in either of the treated patients but the clinical course of the disease changed into a less aggressive form in all cases.

Discussion

The presented data confirmed our previously published assumption on partial therapeutic effect of HuIFN alpha in CAH-B patients (Stanček *et al.*, 1988, 1989). Most apparent were reversed oscilations of HBsAg/HBeAg levels. It was suggested that this effect is possibly mediated through cytolytic activity of LAK/NK cells enhanced by exogenous IFN. Endogenous IFN itself is presumed to be a stable and complex immune function and usually it should not need exogenous IFN support. The question remains open, however, what happens when endogenous IFN is not produced in a proper quality at a proper place and time. Is it then possible to replace it by exogenous IFN? The published data concerning this question are often contraversal. In the case of CAH-B development it is presumed that disfunctions of cellular immune mechanisms, namely LAK/NK cells and cooperating humoral defense factors like IFN and interleukins are most responsible for its chronic development (Vladutin, 1978; Kimoto and Taguchi, 1987; Cauda *et al.*, 1988).

From the collected data presented in this report no conclusive judgement on the therapeutic effect of HuIFN alpha alone or combined with corticoids can be drawn either. The complexity of immune mechanisms amplified by varieties of mutual interrelations make decisive conclusions extremely difficult. In this report we showed that mere quantification of immunity markers

is far not sufficient for more objective estimation of IFN effectiveness. Off some value is the analysis of long-course dynamic follow-ups of properly selected pathogenetic markers. In our clinical trials administration of HuIFN alpha alone or in combination with corticoids partially affected the dynamic of HBV serum markers. It also influenced immunoglobulin levels, PA, PI, LTT-PHA, and IRI values. At least some of these changes may contribute to less aggressive development of the described CAH-B infections following such therapy. More valuable data should still come from the properly checked functional capability of LAK/NK cells, lymphokine interactions and other immune factors. It is also possible that other cells, e.g. macrophages, Kupfer endothelial cells in cooperation with lymphokines including IFN might be involved as already demonstrated for mouse hepatitis virus infection (Prevost *et al.*, 1975; Virelizier, 1980; Pereira *et al.*, 1984*a*, 1984*b*).

Based on our observations transformation capacity of lymphocytes is well preserved but in some VH-B infections there is a defect of necessary stimulation mechanism since such lymphocytes can be fully transformed by *in vitro* tests. Together with a percentage of transformable lymphocytes there is a parallel decrease of OKT 4 number. This can be partially reversed by corticoid introduction which activate HBsAg/HBeAg production. Under these conditions application of exogenous IFN is fully substantiated and usually the most effective.

Our results confirmed that we have to be careful in making clear-cut conclusions on the therapeutic effectiveness of exogenous IFN. The statistical data have some value when large groups of patients are analysed. Evaluation of selected immunological markers together with aetiological and biochemical data in individual patients should give us though more valuable information for objective judgement of the therapeutic potential of exogenous IFN.

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