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EXHAUSTION OF PERIPHERAL BLOOD IMMUNE CELLS IN CHBV AND CHCV PATIENTS: AN EVALUATION OF IFN-ALPHA AND IFN-GAMMA PRODUCTION.

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ABSTRACT

It's common knowledge that, interferons (IFN) are crucial cytokines, which control various physiological processes in many cells. On top of that, they are pivotal players in pathogenesis of many diseases, namely viral and oncological. We have studied spontaneous and induced production of interferons in chronic hepatitis patients. In chronic viral hepatitis C and B patients (CHCV and CHBV), the induced level of IFN-α and IFN-γ production was significantly lower than in the control group, which gives grounds for suggesting that peripheral blood leukocytes are exhausted in patients with chronic viral hepatitis.

KEY WORDS: Interferon, spontaneous production, induced production, viral hepatitis, immune cell exhaustion.

INTRODUCTION

Interferons play a key role in the pathogenesis of viral hepatitis, being pleiotropic with cytokines.[7] [2] [1] IFN-α prevails antiviral, antiproliferative and pro-apoptotic effects, which makes it the first protective line against viruses.[1] While the main functions of IFN-γ are stimulation of macrophages, CD8 + cytotoxic T cells and Th1 cells.[2] Given their important antiviral and immunostimulating properties, IFN drugs have been developed, and they have been widely used in the treatment of viral hepatitis, but their effectiveness has not always justified the side effects.[6] [8] [9] Since many patients remain untreated, there is an urgent need to further studying the pathogenesis, and to obtain new data that will be the basis for developing new methods of diagnosis and treatment.

It is well known that, spontaneous and induced production of cytokines provides valuable information about the functional state of immune cells. Therefore, our laboratory studied spontaneous and induced levels of IFN-α and IFN-γ in patients with CHVC and CHVB. It is known that spontaneous production of cytokines is the production of a specific cytokine by peripheral blood cells *in vitro*, which indicates the *in vivo* activation of immune cells in the context of infection or physiological processes, and was more frequent in regions where infections were endemic.[10]

Induced production of cytokines is the synthesis of cytokines *in vitro* by mitogen activated leukocytes[11], and indicates the preserved potential of immune cells.

Objective: to assess the level of spontaneous and induced IFN-α and IFN-γ production in CHBV and CHCV patients.

MATERIAL AND METHODS

We sampled blood from 29 patients (including 12 men and 17 women) with chronic viral hepatitis (CHCV) (8 CHBV and 21 CHCV) in the department of chronic hepatitis of the NIIEMIS clinic, the Ministry of Health of the Republic of Uzbekistan. Diagnosis was established on the basis of clinical and laboratory studies in the hospital. Clinically, chronic hepatitis manifested by: rapid fatigue (in 41% of patients), weakness (in 62% of patients), decreased appetite (in 65% of patients), pain under the right chest (in 72% of patients), flatulence (in 48% of patients), nausea (in 34% of patients), bitterness in the mouth (in 24% of patients), headaches (in 17% of patients). The laboratory parameters in the patients on the average were as follows: Bilirubin 25.8 ± 2.9 μm / L, AlAt 0.01 ± 0.05 mmol / l, AcAt 0.049 ± 0.02 mmol / l. The patients' blood was taken to a sterile tube, centrifuged for serum collection, and the level of IFN-α and IFN-γ was determined using ELISA kit Vector-Best (manufactured in Novosibirsk, Russia). To determine the induced production of IFN-α and IFN-γ, the blood was incubated at + 37 ° C in a sterile tube with nutrient medium 199 and mitogen (viral antigens and ribosomal fractions of bacteria, respectively) for 24 hours. Incubation conditions for the determination of spontaneous production of IFN-α and IFN-γ differed only in the absence of mitogens. After 24 hours, the tubes were centrifuged, the supernatant was collected and the content of IFN-α and IFN-γ was determined

therein. In parallel, the above manipulations were carried out using the blood of practically healthy individuals, who made up the control group (Table 1). The data obtained were statistically processed using the Microsoft Excel 2016 program, calculating the arithmetic mean (M), the standard deviation (σ), the standard error (m), the Student's criterion (t), and calculating the error probability (P).

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RESULTS AND DISCUSSION

Data obtained on induced, spontaneous and serum levels of IFN-α and IFN-γ production in patients with CHCV and CHVB are provided in Table 1. There was no significant difference between serum IFN-α and IFN-γ levels in the groups.

Concerning spontaneous production, a significant decrease in the spontaneous production of IFN-γ compared with the control group in 5.4-fold ($p > 0.05$) and 11.7-fold ($p < 0.05$) was observed in patients with CHCV and CHBV, respectively. Undoubtedly, this suggests a suppressed potential of immune cells in groups of patients. Nevertheless, this picture rather shows increased spontaneous production in a healthy group, because different processes in the body activate the immune cells, which is reflected in vitro by spontaneous production of cytokines, and this phenomenon is often observed in people living in endemic infections regions.[10]

The analysis of the results also showed that the induced production of IFN-γ was significantly suppressed in the CHVC and CHVB groups, being 8.4 ($p < 0.001$) and 7.4 ($p < 0.001$) times less than the control group, respectively. The synthesis potential of IFN-α tended to decrease more than 2-fold in the groups of patients with chronic viral hepatitis relative to the control group. Consequently, it can be concluded that in patients with CHVC and CHCVV, as expected, the production potential of these interferons is significantly depressed, especially IFN-γ.

In recent years, the interest in immune cells exhaustion has increased, and there are many excellent works describing the molecular basis of their dysfunction.[3] [4][5] For example, with prolonged viral infections plasmatic dendritic cells, which are the main producers of IFN-α, lose the ability to produce IFN-α in large amounts.[12] Moreover, hepatitis B virus polymerase and HBx protein can bind signal proteins of the DDX3 and IPS-1 receptor pattern, thereby inhibiting the synthesis of interferon.[6] Moreover, production of IFN-γ by T cells is decreased in patients with some chronic infections[5], which is reflected in our results. Due to the long-term stimulation of T cells by antigens and cytokines, an increase in the expression of suppressive receptors on their membranes is observed, and the most prominent agent is programmed cell death receptor 1 (PD-1)[4, 5] It should be noted that, experimentally blocking PD-1 led to a significant increase in the production of interleukin-2 and IFN-γ, both in peripheral T-cells and in tissue-resident T-cells.[3] As with many different therapies, the monotherapy of the PD-1 blocker yielded modest results, unlike the combination therapy.[5]

Table 1: Results of serum, spontaneous and induced interferon production.

Cytokine	Type	Control	CHCV	CHVB
IFN-α	serum (pg/ml)	17,85±4,7	12,7±0,3*	15,7±2,1*
	Spontaneous (pg/ml)	14,7±3,5	14,1±0,8*	14,2±1,2*
	Induced (pg/ml)	112,9 ± 38,2	46,5±7,3*	45,9±12,3*
IFN -γ	serum (pg/ml)	14,91 ±1,85	12,4 ±1,6*	8,2 ±2,7*
	Spontaneous (pg/ml)	224,26 ±86	41,4 ±21,6*	19,05±5,3**
	Induced (pg/ml)	1310,68 ±124,24	154,8 ±67,3***	176,7 ±66,2***

Reliability in comparison with control group *(p>0,05);**(p<0,05);***(p<0,001)

CONCLUSION

Analysis has showed that in patients with CHCV and CHVB, the induced level of IFN-α and IFN-γ production were profoundly decreased in comparison with the control group, which gives grounds for assuming the exhaustion of peripheral blood leukocytes in patients with chronic viral hepatitis. As the exhaustion of immune cells is an pivotal problem not only for chronic viral diseases, but also for oncology, the urgent need to develop complex diagnostics and effective therapy in the future is emphasized.

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