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INDUCTION OF GAMMA-INTERFERON: THE POSSIBILITY OF USING RIBOSOMAL AND MEMBRANE FRACTIONS OF BACTERIA AS A MITOGEN

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SUMMARY

The study of induced cytokine production is one of the important and developing aspects of establishing the immunological state of the organism in the modern world. But due to the inaccessibility of classical mitogens remains poorly understood. The aim of the study was to identify the possibility of using the complex of ribosomal and membrane fractions of the bacteria Klebsiella pneumoniae, Streptococcus pneumoniae, Streptococcus pyogenes and Haemophilus influenzae to induce the production of gamma-interferon. The results showed the possibility and feasibility of their use as a mitogen in patients with chronic viral hepatitis B and C.

KEY WORDS: Gamma-interferon, induction, ribosomal and membrane fractions.

In laboratory diagnostics, in addition to determining well-known serum levels, "spontaneous" and "induced" cytokine production is also investigated. As we know, the level of cytokine concentration in serum or plasma reflects the current state of the immune system, "spontaneous" production indicates how much blood cells have already been activated in vivo[3], and "induced" - allows us to assess the potential ability of cytokine induction.[4]

Induction of cytokine production is carried out using mitogens. Mitogens are substances of a protein nature, which, after binding to a target cell, initiate the process of mitosis in it. Phytohemagglutinin (PHA) and concanavalin A (CoA) are classical mitogens.[1]

Recently, there has been a growing interest in the world to study the effect of gamma-interferon (γ-INF) on the hepatitis C virus (HCV). In the body, in response to the introduction of HCV, cytolytic and non-cytolytic mechanisms of inhibition of its replication are activated, and γ-INFA triggers precisely non-cytolytic mechanisms.[5] γ-INF is produced only by activated T-lymphocytes and natural killers.[2]

So, it becomes obvious the importance of studying different aspects of the production of γ-INF for a comprehensive understanding of the body condition of patients with chronic viral hepatitis B and C. But due to the limited commercial kits for the induction of this kind of research is practically not carried out. In addition, more than 100 years have passed since the first use of mitogens, and new types have not been developed. In this study, we tried to induce the production of γ-INF with ribosomal and membrane fractions of bacteria and compare them with classical mitogens.

THE AIM OF THE STUDY

Identify the possibility of using the ribosomal and membrane fractions of the bacteria Klebsiella pneumoniae, Streptococcus pneumoniae, Streptococcus pyogenes and Haemophilus influenzae as a mitogen to induce production of γ-INF.

MATERIALS AND METHODS

We studied the sera of 41 patients with chronic parenteral viral hepatitis who did not receive antiviral therapy. Of these, in 17, γ-INF induction was performed using a mitogen containing PHA and CoA (Test 1), in serum of 24 patients, induction was performed by using the ribosomal and membrane fractions of Klebsiella bacteria pneumoniae, Streptococcus pneumoniae, Streptococcus pyogenes and Haemophilus influenzae (Test 2).

Clinically chronic hepatitis was manifested: fatigue (in 41% of patients), weakness (in 62% of patients), loss of appetite (in 65% of patients), pain in the right upper quadrant (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). Laboratory indicators in patients on average

were as follows: Bilirubin - 32.76 ± 7.70 μm / l, AlAt - 1.09 ± 0.08 mmol / l, AsAt - 0.53 ± 0.04 mmol / l.

The control group included 6 practically healthy people (3 of them are men and 3 women). The concentration of γ-INF was determined by enzyme immunoassay (ELISA), commercial reagent kits Vector-Best (Novosibirsk) in the Laboratory of Fundamental Immunolgy RNIC MZ RU. The level of serum γ-INF was determined immediately after the release of serum.

The method of determining the spontaneous and induced γ-INF products was carried out according to the instructions of the commercial set of the Vector-Best "Cytokine-Stimul-Best".

Test 1: Whole blood, both of the control and experimental group, was diluted in "Nutrient medium 199" in a 1:4 ratio (spontaneous production), 1 ml of the diluted sample was taken from the same tube for mitogen induction containing PHA and CoA from the kit;
Test 2: Whole blood, both of the control and experimental groups, was diluted in "Nutrient medium 199" in a 1:4 ratio (spontaneous production), 1 ml of the diluted sample was taken from the same tube for mitogen induction containing ribosomal and membrane fractions of bacteria.

Indicators of spontaneous and induced γ-INF production were determined after 24 hours of incubation at 37 ° C in a thermostat.

The obtained data was statistically processed in Microsoft Excel 2013 by calculating the arithmetic mean value (M), standard deviation (σ), standard error (m), Student's criterion (t) with the calculation of the error probability (P). The results were compared with the corresponding indicators of the control group.

RESULTS AND DISCUSSION

In Test 1, the average statistical indicators of the γ-INF concentration in the experimental group were as follows: serum - 7.45 ± 0.45 pg / ml (control - 1.86 ± 0.21 pg / ml); after incubation: spontaneous - 17.84 ± 0.55 pg / ml (control - 2.01 ± 0.19 pg / ml); induced - 18.51 ± 0.90 pg / ml (control - 7.64 ± 1.35 pg / ml). (Table 1)

Table 1. Comparative table of results Test 1 *(unit of measurement pg/ml).*

	Test	Control
Serum	7,45±0,45*	1,86±0,21
Spontaneous	17,84±0,55*	2,01±0,19
Induced	18,51±0,90*	7,64±1,35

*Note: * - the data are statistically reliable (p<0,001)*

In Test 2, the average statistical indicators of the γ-INF concentration in the experimental group were as follows: serum - 11.24 ± 1.15 pg / ml (control - 1.86 ± 0.21 pg / ml); after incubation: spontaneous - 36.85 ± 8.22 pg / ml (control - 2.01 ± 0.19 pg / ml); induced - 218.75 ± 53.40 pg / ml (control - 1310.68 ± 124.24 pg / ml). (Table 2)

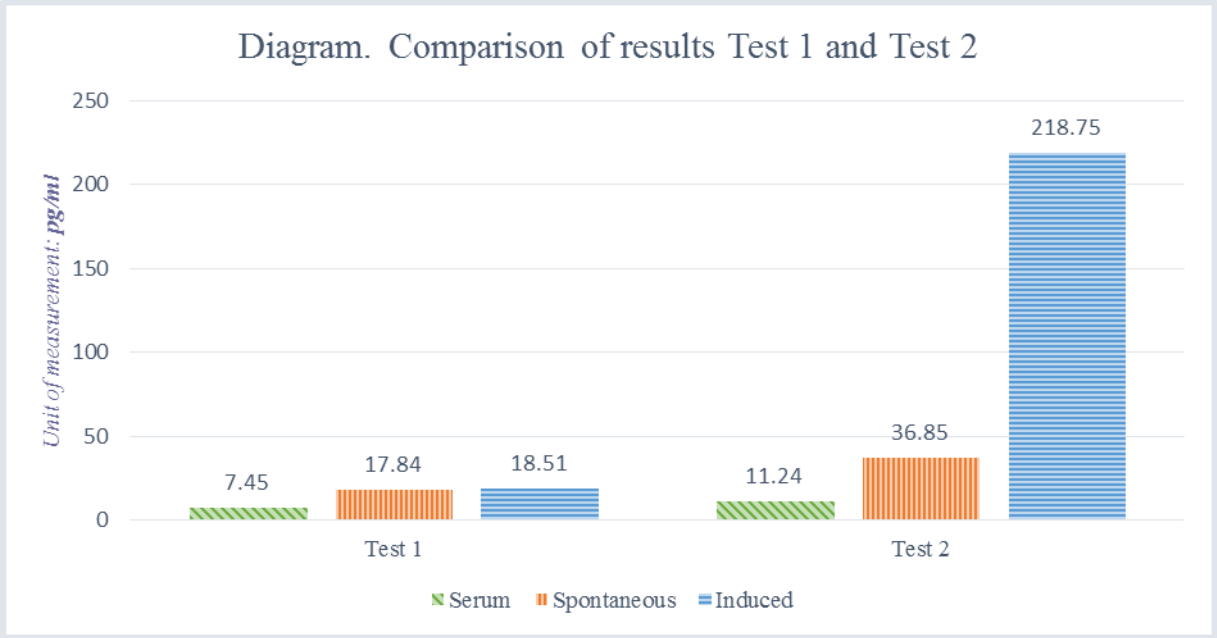
Table 2. Comparative table of results Test 2 *(unit of measurement pg/ml).*

	Test	Control
Serum	11,24±1,15*	1,86±0,21
Spontaneous	36,85±8,22*	2,01±0,19
Induced	218,75±53,40*	1310,68±124,24

*Note: * - the data are statistically reliable (p<0,001)*

As we know, the level of induction is determined depending on the difference between spontaneous and induced products, and the higher this difference, the stronger the induction was. The results of induced γ-INF production in patients with chronic viral hepatitis do not exceed the indices of spontaneous production. The control group induced products by 3.8 times higher than the indicators of spontaneous products. The results obtained in Test 1 showed that classical mitogens containing PHA and CoA are not advisable for induction of γ-INF in patients with chronic viral infections. We believe that this is due to the relatively low ability of PHA and CoA to induce γ-INF production.

In test 2, we used mitogen containing ribosomal and membrane fractions of bacteria to induce γ-INF. Despite the fact that in the control group, the index of induced production is much more than that of the experimental group, attention should be paid to the difference between spontaneous and induced production in patients themselves. In patients, the indices of induced production are 5.9 times higher than the indices of spontaneous production. Against the background of the lack of obvious induction by the classical mitogens of PHA and CoA, this indicator stands out positively. And the interpretation of test results is changing dramatically. (Diagram)



In vitro studies of the patients' condition and interpretation of the results have always been a challenge for specialists and scientific and practical medicine. In addition, to give a correct assessment of the potential of the organism itself often fails due to the not entirely correct approach to the analysis.

After comparing our results, we came to the conclusion that, despite the reduction in the cell potential to produce γ-INF in patients (relatively healthy), it still remains and appropriate in vitro studies should use appropriate mitogens to induce a particular type of cytokine. Based on the results, it is possible to judge that the ribosomal and membrane fractions of bacteria can be used as a mitogen in in vitro studies to induce the production of γ-INF.

ABSTRACT

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