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THE STUDY OF ESCHERICHIA COLI INTERFERON-PRODUCING ABILITY

The work provides experimental research of bacteria Escherichia coli interferon producing ability. The dynamic accumulation of interferon in growing cells and organ cultures of mice was studied. It is shown that introduction Escherichia coli in the serum and organ cells of mice causes formation of endogenous interferon which is capable to protect of mice from a lethal virus infection. Experiments were undertaken for the purpose of expansion of a spectrum of studies on immunological mechanism development during the interaction with bacteria and viruses and for data acquisition about interferon reactions of organism in response to action intestinal of bacteria Escherichia coli.

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Introduction

The fight against infectious diseases is one of the priority directions of practical public health. Currently, the attention of clinical infectious physicians has involved a new, alternative direction in treatment and preventive maintenance of infectious diseases. Development in this direction became possible thanks to opening of cytokines - cellular factors of immunity and products of their interaction. The most known preparations of cytokines are interferon and it inducers, interleukins, the tumour necrosis factor, etc. Their applications are successful thanks to a wide spectrum of their action: anti-inflammatory, antibacterial, antiviral, antineoplastic, radioprotective, etc.

Differentiated use of cytokine preparations promotes activation and normalization of immune regulatory processes, improving ability of an organism to protect against an infection. Interferon as one of cytokines, is the original marker defining a current and the forecast of an infectious disease (Onishchenko, 2002; Ershov and Kiselev, 2005; Shmeleva 2008; Omarova et al., 2007; Haitov and Pinegin, 2000). It is known that is opportunistic-pathogenic bacteria Escherichia coli carry out an antagonistic functions in relation to pathogenic intestinal bacteria: allocate substances colicins, enzymes, tumor necrosis factor, interleukins, etc. (Bondarenko, 2009; Kelly et al., 2005; Steele and Fidel, 2002).

In the current literature there are not enough data about interaction of bacteria with cells of an intestinal path. The purpose of our researches included studying of ability of opportunistic pathogenic bacteria Escherichia coli to production of interferon in various organ of white mice.

Materials and methods

Bacterial strains and laboratory animals

Locally circulating strains of the bacteria Escherichia coli were isolated from patients. Inactivated by heating the bacterial preparations were prepared from opportunistic strains of Escherichia coli of the Enterobactericeae family.

Then the strains were selected using the standard test for absence of toxigenic properties. White mice with weight of 25-30g were used in the study.

Organ cultures

The preparations of organ cultures of mice: liver, spleen, lungs, heart, brain and peripheral blood serum were prepared (Irzhanov and Hesin, 1977). 109 microbic bodies E. Coli were entered intraperitoneally to mice (24 heads). The mice were hammered in 3, 5, 24, 48, 72 and 96 hours after E.coli administration; there were received blood, liver, spleen, lungs, heart, brain, then crushed in a mortar, weighed on scales and prepared suspension in cultural liquid media. Suspensions were centrifugated at 1000 rpm within 20 minutes. Supernatant liquid was separated and activity of interferon in it was defined.

Cultures of cells

There were used cultures of growing cells of the mouse (L929) and human (RD) for an interferon induction. The same growing cells were applied for quantitative definition of interferon in other materials: serum of mice, in supernatant liquids from the growing and lymphoid cells. Culture of survival mouse cells L929 and human cells RD were cultivated on RPMI 1640 media or on double minimal essential media (DMEM) "Eagle". The growth components - 10% fetal serum, 2 mM L-glutamine ("Sigma") and 100 μg/ml gentamycine ("Pan-Eko", Moscow) were added. Cell cultures infected with the virus were maintained in the same medium with 2% fetal bovin serum (FBS). Working concentration of cells was fixed within $4\text{-}5x10^5$ cells/1 ml. Cells were removed from the surface of the bottles by 0.02% Versen solution. Cells were placed in the thermostat at the temperature 37º C in atmosphere 5 % CO_2 (Andzhaparidze et al., 1962).

Determination of the quantity of interferon

Interferon was defined by a micromethod in a monolayer of cells L_{929} and RD titration in 96-well plastic panels (Novohatskij et al., 1978). For this purpose investigated material was put on well with cellular monolayer. The interferon, induced by the Newcastle disease virus, was used as a standard reference-preparation α/β. The interferon, induced by staphylococcal enterotoxin A, was used as a reference-preparation γ. In the presence of interferon there was an inhibition of destructive (cytopathogenic) action of a virus on cells and cells remained live. Interferon titer was considered the highest dilution of interferon-containing material causing 50% suppression of cell destruction under the influence of encephalomyocarditis virus of mice in comparison with control - in pure inoculate cultural fluid.

Serum of mice was received through certain time intervals after intraperitoneal introductions of 10^9 microbic bodies/ml of preparation E.coli. Samples of serum were frozen in low-temperature freezer and stored until defined in it activity of interferon.

It was studied the ability E.coli and a traditional Newcastle disease virus to induce production α/β - interferon on RD-cells. For this purpose in the cell culture which was growing up in 24-wells plastic panels there was added: either E.coli (5-10 microbic bodies/1 cell, or a virus of illness Newcastle - 5 units (50% of cytopathogenic effective doses/1 cell). Panels grew within 18-24 hours in incubation medium. After that the incubation medium from wells was collected and interferon activity in it was defined.

For this purpose we prepared the culture of lymphoid cells from a spleen of mice. Splenocyte suspensions with concentration of 10^6 cells/1 ml grew up in the DMEM media, added with 10% fetal serum. Suspension were placed in penicillin bottles on 1.0 ml, then brought either (1) 100 μl E.coli in concentration of 107 microbic bodies/ml or (2) 200 μl of the Newcastle diseases virus - 10^4 of 50% of effective infectious doses/μl; or (3) 100 μl staphylococcal enterotoxin A - 10 μg/ml. Hanks's salt solution were put in the control bottles. Interferon activity was measured in samples of culture liquid after after the 24-hours (for E.coli and the Newcastle diseases virus) and the 72-hours (for staphylococcal enterotoxin A).

After extraction in aseptic conditions a spleen of mice carefully washed from blood with penicillin addition (100 units/ml). Then it was crushed to slices up to 2-3 mm. The

received organ culture was distributed in regular intervals in sterile 10-ml bottles. Further experience was organized according to a method, described in the previous section.

Influence of low pH values and warming up on properties of interferon was studied in the E.Coli-induced serum of mice. Group of animals (10 animals) was inoculated intraperitoneally by 10^9 E.coli microbes. The second group of mice (10 animals) was administered by 10^6 of 50% cytopathogenic doses of Newcastle disease virus. Third group of mice (10 animals) was inoculated intraperitoneally with 100 micrograms of staphylococcal enterotoxin A. 5-6 hours after injection the blood of animals was taken; serum was separated to measure the interferon activity. Part of received interferon-containing serums was processed by 0.1 N HCl (pH 2) and maintained at +4º C within 18-24 hours. pH environment was restored to 7.2 through adding 0.1 N NaOH and activity of interferon was defined. Another portion of serum samples was warmed up in the ultrathermostat at 56ºC within 30 minutes, then activity of interferon was measured in them.

Two groups of mice (on 20 heads in everyone) participated in the study. The first group of animals was intraperitoneally entered 10 of 50% lethal doses of encephalomyocarditis virus of mice. The control group of mice was administered with 1.0 ml of a physiological solution. 8 hours later animals of both groups were entered intraperitoneally 10 of 10^9 microbial bodies. The animals were observed during 15 days with daily recording of dead animals. Statistical processing of the received data was made according to a standard method (Ojvin, 1964).

Results of research

Kinetics of accumulation of endogenous interferon-induced E.coli in various organs of white mice

Table 1 shows that the interferon was found in all organs. Activity of interferon in serum was significantly higher than in the organs. The content of interferon in suspensions prepared from organs was different. Thus, the highest level was in the liver (67.9±2.5 Unit/ml) and spleen (31.4±0.8 U/ml), and the lowest content of interferon found in brain tissues of mice (8.3±1.4 U/ml).

TABLE 1. QUANTIFY OF A/B ENDOGENOUS INTERFERON IN VARIOUS ORGANS IN MICE DEPENDING ON TIME OF BLOOD SAMPLING

The investigated organ cultures	Activity of interferon (a unit / ml) in various terms of a blood sampling and bodies after introduction preparation E.coli (in hours) (M±m) to mice*					
	3	5	24	48	72	96
Liver	17.7±1.8	67.9±2.5	16.3±2.1	10.4±0.9	7.4±0.8	<2
Spleen	17.0±1.7	31.4±0.8	20.5±1.2	14.5±0.4	3.8±0.7	<2
Lungs	5.6±0.9	10.7±1.3	11.8±1.3	5.2±1.4	4.2±0.8	<2
Cor	7.5±1.1	15.0±2.0	13.4±2.4	5.1±1.1	3.6±0.7	<2
Brain	5.7±1.2	12.3±1.4	11.5±1.1	10.3±0.9	8.4±0.5	<2
Peripheral blood	28±4.6	256.4±18.5	26.6±12.4	<2	<2	<2

Notes: * P<0.05

The maximum amount of interferon in the bodies, as well as in serum, was noted after 3-5 hours post administration of E. coli. Interferon in the organs was detected over a longer time (48-72 hours) than in the peripheral blood, where its circulation stopped in about 24 hours after administration.

Study of interferon induction activity of E.coli in cultures of somatic- and lymphoid cells

The first series of experiments were focused to study of the ability of E.coli and Newcastle disease virus to induce production of α/β-interferon in cultures of survival cells RD. In incubation medium of cells, treated by Newcastle disease virus, the interferon

activity reached 345±35.4 U/ml. α/β-interferon was not found in cultures of the somatic cells RD processed by E. coli. Thus, in our experiences E.coli did not induce production of β-interferon in the somatic human cells RD cells. In the second series of experiences we studied ability of E.coli, Newcastle disease virus and staphylococcal enterotoxin A to induce interferon production in lymphoid cells. Interferon was not found in the incubation medium with E.coli; while the Newcastle disease virus and staphylococcal enterotoxin A in the culture of splenocytes induced interferon with antiviral activity 136±20.1 U/ml and 95±18.2 U/ml, respectively. Interferon was not found in control bottles.

Quantify of interferon production induced by E.coli in organ cultures of mouse spleen

Table 2 shows the titers of interferon produced depending on the application of various inducers. The titers indicate on interferon production in organ cultures of mouse spleen under the action of E.coli, Newcastle disease virus and staphylococcal enterotoxin A.

TABLE 2. PRODUCTION OF INTERFERON IN ORGAN CULTURE MOUSE SPLEEN

Interferon inducers	Activity of interferon (U/ml) induced in culture mouse spleens (M±m)*
E.coli	21±2,2
Newcastle disease virus	24±8,4
Staphylococcal enterotoxin A	22±8
Control	0

Notes: * P<0.05

The effect of low pH environment and temperature processing on on the antiviral properties of interferon-induced E.coli

It is thus established that activity of interferon in control serum of the mice received E.coli made accordingly: 251±36.5 ed/ml, 296±32.8 ed/ml and 96±17.4 ed/ml of interferon. Activity of interferon induced by E.coli and Newcastle disease virus, remained virtually stagnant after the described processing; while the activity of γ interferon induced by staphylococcal enterotoxin A completely disappeared (Table 3). Low level of pH media and temperature processing slightly reduced the antiviral activity of E.coli-induced interferon. Thus, the specified physical influence has not rendered appreciable influence on antiviral property of the interferon induced by E.coli and the Newcastle disease virus.

TABLE 3. STABILITY OF INTERFERON, INDUCED BY E.COLI, NEWCASTLE DISEASE VIRUS AND STAPHYLOCOCCAL ENTEROTOXIN A, TO LOW VALUES OF PH AND HEAT

Interferon inducers	Control (before processing)	Activity of interferon (U/ml) in serum after processing: (M±m)*	
		pH 2	T 560C
E.coli	251±36.5	122±27.6	114±28.4
Newcastle disease virus B	296±32.8	245±31.4	218±24.8
Staphylococcal enterotoxin A	96±17.4	0	0

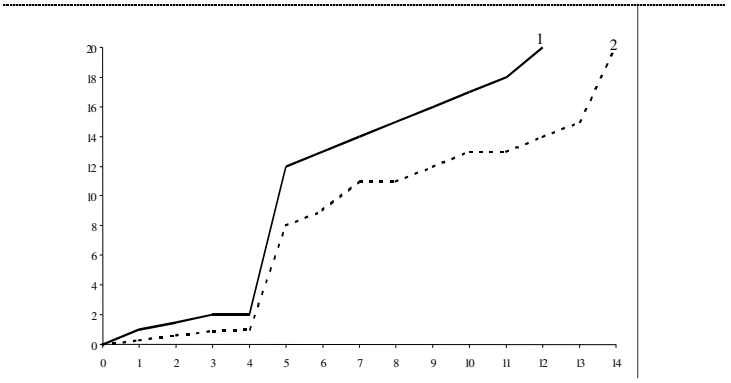
Notes: * P<0.05

Influence of E.coli on a virus infection course in mice

During the experiment it was found that the experimental group pretreated with E.coli the in 13 goals from 20 lost during 14 days; while in the control group who did not receive E.coli all mice died. Thus, mortality from viral infection in the experimental group was less than in the control group of animals; morbidity and mortality from the encephalomyocarditis virus were more manifested in the control group.

Thus, the bacterium E.coli induces the production of both acid-labile alpha and heat-labile interferon gamma, rendering a protective effect in mice against 100 lethal doses of encephalomyocarditis virus of mice.

FIGURE 1. CUMULATIVE MORTALITY OF ANIMALS FROM ENCEPHALOMYOCARDITIS VIRUS OF MICE



Notes: 1 - control animals; 2 - mice, that received E. coli

Discussion

It is currently expanding the spectrum of research on the study immune mechanisms of interaction of infectious agents - the bacteria's and viruses with organism. There is a wide range of specific vaccines, serums, antibiotics and chemotherapy drugs used for medical treatment and prevention of infectious diseases. However, they have some significant damage: the use of vaccines and serums is limited to wide range of pathogenic bacteria and viruses. 4th generation antibiotics from the cephalosporin order, though being highly effective, cause severe dysbacterioses in the normal intestinal microflora (Onishchenko et al., 2002). Interferon, being low-molecular protein - a cytokine, plays an important role in genetically programmed immune system modulation of the body, carried out at the cellular level, through triggering mechanisms for the protection of all damaging factors acting on the organism: bacterial infections, toxins, radiation and etc. It is known that interferon inducers are natural or synthetic origin (Ershov et al., 2005).

We have obtained data on active immunostimulatory functions of interferon induced by locally circulating strain of opportunistic bacteria Escherichia coli. It is shown that interferon is not detected in cultures of somatic and lymphoid cells of mice after induction of E.Coli.

References

Andzhaparidze, O., Gavrilov, V., Semenov, B., Stepanova, L., 1962. Culture of cells in virologic researches [Kul'tura tkani v virusologičeskih issledovanijah], in Russian, Moscow: Medgiz

Aspetov, D., Alshinbaeva, G., Aspetov, R., Omarova, M., Zhumatova, B., 2007. "Application of Kazakhstan immunobiologic preparation of interferon inducer in complex therapy against chronic virus hepatitis B and C," proceedings of international conference "Virus hepatitises: Achievements and prospects" [Virusnye gepatiti: Dostizhenija i perspektivi], in Russian, Astana, October 4-6, pp.41-43

Aspetov, D., Fedotov, E., Aspetov, R., Nygmanova, G., Zhumatova, B., 2002. "Therapeutic efficiency of improved bacterial liquid interferon inducer - for a labial and sexual herpes," Hygiene, epidemiology and immunobiology, Almaty, Kazakhstan, No.1-2, pp.65-68

Bondarenko, V., Lihoded, V., 2009. "Interaction of intestinal flora with Toll-like receptors in norm and a pathology," Immunology [Immunologiya], in Russian, Vol.30, No.3, pp.317-20

Ershov, F., Kiselyov, O., 2005. Interferon and theirs inducers (from a molecule to medicines) [Interferon i ih induktory (ot molekuly do lekarstv)], in Russian, Moscow: Geotar - Media

Haitov, R., Pinegin, B., 2000. "Modern representations about protection of organism protection against infections," Immunology [Immunologiya], in Russian, No.1, pp.611-65

Irzhanov, S., Hesin, J., 1977. Organ cultures in virological research [Organnye kul'turi v virusologicheskih issledovaniyah], in Russian, Moscow, Medicine

Kelly, D., Conway, S., Amirov, R. 2005. "Commensal gut bacteria: Mechanism of immune modulation," Trends immunol., Vol.26(6), pp.326-33

Novohatskij, A., Cherkashina, N., Ershov, F., 1978. "The accelerated method of interferon determination in experimental infection," Virology Issues [Voprosi Virologii], in Russian, No.3, pp.303-306

Ojvin, I., 1964. "Statistical processing of results in experimental research," Patol. Physiol and Experimental Therapy [Patologicheskaya Fiziologiya i Experimentalnaya Terapiya], in Russian, No.4, pp.76-85

Onishchenko, G., Aleshkina, V., Afanaseva, S., Pospelov, V., 2002. Immune biological preparations, application prospects in infectology [Immunologičeskie preparaty i perspektivy ih primenenija v infektologii], in Russian, Moscow, GOU VUNMC, Ministry of Healthcare of the Russian Federation

Shmeleva, E., Vartanjan, R., Aleshkin, V., Paramonova, J., Baturin, I., Malyshev, N., 2008. "Prospects of use of preparation from Corynebacterium for preventive maintenance treatments of respiratory infections," Bulletin of the Russian Academy Sciences [Bulleten Rossiyskoy Akademii Nauk], in Russian, No.3, pp.12-15

Steele, C., Fidel, P., 2002. "Cytokine and chemokine production by human oral and vaginal epithelial cells in response to Candida albicans," Infect and Immun., Vol.70(2), pp.577-83