8. INFECTIOUS IMMUNOLOGY AT THE PRESENT STAGE

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CREATION OF THE IMMUNOFERMENTIC TEST SYSTEM FOR DETECTING C3 COMPLEX COMPONENT WITH THE USE OF PEPTIDOGLYCAN

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Any inflammatory process is accompanied by activation of the complement system, as well as increased production of the acute phase protein — C3. C3 binds to the surface membrane of the bacterial cell and enhances the formation of new C3b. It is known that corynebacteria colonize all the mucous open cavities of a person, and their metabolites play a role in the system of immunity.

The aim of the study was creation of an ELISA system for participation in C3 activities due to its ability to bind to peptidoglycan corynebacteria.

The ELISA method offers sorption in the wells of a micro-panel of peptidoglycan. Then, a solution containing a human complement component C3 with unknown activity is introduced into the wells. The incubation is carried out in the presence of EDTA to block all pathways of complement activation. C3 binds to the sorbed peptidoglycan, followed by removal of the onion content and introduction of the enzyme conjugate with antibodies against the human C3 component, washing out the unbound conjugate, introducing the substrate of the conjugated enzyme, and calculating the components of C3 by the amount of the product of the enzymatic reaction.

The kit contains a flat-bottomed micro-panel with sorbed peptidoglycan, a conjugate combined with antibodies to C3 components as a standard. The incubation takes place in the presence of EDTA.

Use of the proposed test system to identify the identified deficiencies in the blood serum of patients with ENT pathology (bronchitis, tonsillitis, sinusitis, otitis) is determined by the increased content of C3 complement components in comparison with its content in the sera of healthy individuals by 2 times.

Obtained data that with ENT — pathology in blood serum of sick people there is activation of C3, responsible for the subsequent launch of the entire complement system.

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FEATURES OF THE SUBPOPULATION COMPOSITION CYTOTOXIC T-LYMPHOCYTES IN CHILDREN WITH CONGENITAL CHRONIC HEPATITIS B

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Hepatitis B is an infectious disease caused by the hepatitis B virus, which has a high tropicity to hepatocytes and is capable of transitioning to a chronic form (HBV). The highest frequency of chronization is in children, especially with perinatal infection (up to 90%). An effective mechanism for protecting the body from viral infections is the development of a cytotoxic immune response, during which naive cytotoxic T lymphocytes proliferate and undergo several stages of differentiation, acquiring the ability to kill infected cells. In congenital CHB, the development of an ineffective variant of such a response in children can be a consequence of both immaturity of their immune system and disorders during its formation in the presence of the virus. The determination of the ratio of groups of cells at different stages of this differentiation can contribute to the study of the mechanisms of this pathology.

Purpose of the study was to evaluate the effectiveness of the formation of effector cytotoxic T-lymphocytes in children with chronic hepatitis B acquired due to perinatal infection.

The material of the study was peripheral blood of 9 children diagnosed with CHB, after perinatal infection and without severe accompanying pathologies, as well as 6 conditionally healthy children, aged 7 to 14 years. The following populations of cytotoxic T-lymphocytes ($CD45^+/CD3^+/CD8^+$) were analyzed by multicolor analysis on a BD FACS Canto II device: naive ($CD45RA^+/CD62L^+$), central memory cells (CM, $CD45RA^-/CD62L^+$), effector memory cells (EM, $CD45RA^-/CD62L^-$) and "terminally differentiated" EM (TEMRA, $CD45RA^+/CD62L^-$).

In the blood of the patients studied, a significant decrease in the absolute amount of cytotoxic T lymphocytes was found: 0.41 (0.33–0.60) 10^9/L versus 0.64 (0.48–0.76) 10^9/L in healthy subjects, $p = 0.0496$; the tendency to decrease the absolute number of TEMRA cells is 0.06 (0.05–0.09) 10^9/L versus 0.16 (0.08–0.27) 10^9/L in healthy and relative number of cells — 3.5 (2.6–4.5)% versus 6.6 (3.6–11.2)%. Also, there were no differences in the number of naive cytotoxic cells — 0.22 (0.16–0.29) 10^9/L and 10.2 (8.5–13.9%) versus 0.25 (0.23–0.28) 10^9/L and 10.6 (9.6–12.2)%, the central memory cells — 0.03 (0.02–0.05) 10^9/L and 1.6 (1.2–2, 4)% versus 0.04 (0.04–0.06) 10^9/L and 1.9 (1.5–2.5)% in healthy and effector memory cells — 0.10 (0.05–0.0, 17) 10^9/L and 4.2 (2.7–8.8)% vs. 0.14 (0.08–0.21) 10^9/L and 5.6 (3.9–9.0)% in healthy.

As a result of the study, it was shown: in the peripheral blood of children with congenital CHB, absolute amounts of cytotoxic T-lymphocytes were reduced; there is a tendency to decrease absolute and relative amounts of "terminally differentiated" cytotoxic T-lymphocytes, which may indicate the depletion of this pool of cells due to migration to the injured organ, or a violation of their formation. At the same time, there are no pathologies in the content of naive cytotoxic cells and memory cells.

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INFLUENCE OF MONOSTRAIN AND MULTISTRAIN AUTOPROBIOTICS ON MICROBIOTA AND IMMUNITY OF RATS WITH INTESTINAL DYSBIOSIS

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The aim of the study was to find the differences in autoprobiotics effects on intestinal microbiocenosis and immune system of rats with antibiotic associated dysbiosis.

Intestinal dysbiosis of male Wistar rats was induced by ampicillin and metronidazole. Indigenous enterococci (group E), lactobacilli (group L), bifididobacteria (group B) were isolated from feces before the antibiotic usage and then separately or as mixture of three strains (group M) were given to the animals for 4 days. Rats from control group 1 (C1) didn`t receive autoprobiotics. Animals from control group 2 (C2) didn`t receive antibiotics and autoprobiotics. The study of fecal samples, collected on 4th and 9th days of experiment, was performed by RT-PCR and by metagenome 16S rRNA analysis. The cluster differentiation of lymphocytes was analysed using flow cytometry.

Dysbiosis is characterized (on 4th day) by excessive abundance of filum *Gammaproteobacteria*, *Proteus* spp. (Pr) and, *Klebsiella* spp. (K), and decrease of *Faecalibacterium* sp. (F), *Prevotella* spp. (Pv), *Bacteroides* spp., *Lactobacillus* spp. populations. The decrease abundance of opportunistic enterobacteria was minimal in groups C1 and M. Low efficacy against Pr and K, main decrease of *Lactobacillus* spp. and *Paraprevotella* spp. content in M group coincided with maximum shifts in cluster differentiation of lymphocytes: increase of B-cells, NK-cells, decrease of T-cells and $CD3^+CD8^+$ T-lymphocyte. Surprisingly no significant changes in the immunogram of rats from the group C1 could be detected.

Indigenous enterococci stimulated growth of bacteroides and inhibited growth of lactobacilli and Pv. This autoprobiotic demonstrated low antagonistic activity against Pr. Indigenous lactobacilli inhibited the growth of the Pr and restored the number of Pv. and F. Significant decrease of both K and Pr percentage abundance and the increase of F were observed in group B.

Changes in the composition of microbiota correlated with changes of immunity in different experimental groups. The increase in the abundance of F correlated with the increase of $ThCD3^+$.

$CD25^-FoxP3^+$ content in the spleen in groups L and B. It was found that content of Th $CD44^+62L^+$ lymphocytes in the blood, which was reduced in group B and E inversely correlated with the abundance of *Gammaproteobacteria*.

It should be noted that implementation of autoprobiotics besides the influence on microbiota have a significant effect on immunity, which varies depending of the type of autoprobiotic. The mechanisms of immunomodulatory effects of autoprobiotcs are not yet clear and require further studies.

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POTENTIAL INFLUENCE OF IMMUNOMODULATORS ON THE PRODUCTION OF INTERFERON-GAMMA AND INTERLEUKIN-10 IN LABORATORY ANIMALS VACCINATED AGAINST PLAGUE

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The improvement of specific prophylaxis of infectious diseases involves the search for new highly effective immunomodulators to influence the activation of the factors of active and adaptive immunity. As potential components that increase the effectiveness of live plague vaccine, polyoxidonium and Ingaron (Interferon gamma human recombinant), preparations with a stimulating effect on immune system are of some interest.

The comparatives analysis of polyoxidonium and Ingaron effect on interferon-gamma and interleukin-10 production in BALB/c mice when immunized with culture of *Yersinia pestis* vaccine strain EV line NIIEG were studied.

200 BALB/c mice were subcutaneously immunized with a *Y. pestis* EV NIIEG culture at dose of 2.5×10^4 (group 1), in combination with polyoxidonium at dose of 4 μg (group 2), or with Ingaron in dose of 150 IU (group 3), intact mice (group 4) served as controls. The content of cytokines in blood was determined by enzyme immunoassay on the 3rd, 7th, 21st and 90th days after injection of preparation using commercial test systems (eBioscience, Austria).

On the 3rd day of the experiment, significant increase in the level of both interferon-gamma and interleukin-10 was established in all animals of experimental groups (1 — 58.3 and 29.0; 2 — 57.2 and 65.9; 3 — 83.2 and 45.6 pg/ml, respectively), compared with intact mice (26.2 and 11.1 pg/ml). An increase in the level of cytokines by 3–4 times was noted in the experimental group at 7 and 21 days after immunization. A significant decrease in the amount of interferon-gamma (1 — 48.4; 2 — 35.6 and 33.2 pg/ml in 3 group) was showed after 3 months, but it remained high compared to control (16.3 pg/ml). Content of interleukin-10 in blood of group 1 and group 3 mice decreased sharply by 90th day of observation (to 16.5 and 12.7 pg/ml), while in group 2 remained at high level (34.6 pg/ml), which indicates a possible prolonged action of polyoxidonium on production of cytokines — biomarker for anti-plague immunity.

Thus, potentiating effect of immunomodulators in laboratory animals vaccinated against plague has been established.

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NEUTROPHIL/LYMPHOCYTE DISBALANCE AS A PREDICTOR OF VAGINITIS

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Infectious vaginosis or cervicitis is a frequent cause of ginecologist's appointment. Vaginitis is vaginosys of bacterial, viral or fungal etiology. During this inflammation vagina or cervix surface leucocytes count increases significantly. We were interested how white blood cells (WBC) count changes in peripheral blood during vaginitis and/or cervicitis.

The aim of the study was to estimate absolute and relative WBC count in peripheral blood from patients with and without vaginitis.

A total of 26 (mean age 40±19) women visited IDC ginecologist between 28 April to 12 June 2018. Informed agreement was received from all patients. Blood samples were collected in 5 ml K3 EDTA Vacuette tubes. Alifax Roller was used to erythrocyte sedimentation rate (ESR) determination, Sysmex XN was used to complete blood cell count (CBC) determination. The ginecologic slides were heat-fixed and stained with azur-eosine.

Microscopic exam of normal cervix slides shows not more 15 leucocytes in field-of-view (fov) at 1000 total magnification. Normal vagina slides contains 0–10 leucocytes in fov. Peripheral WBC rates was graded according to age. So we divided patients into 4 groups: with normal leukocyte count in the blood and urogenital tract (n = 10), with normal WBC in blood and elevated WBC in cervical and/or vaginal slides (n = 8), with normal blood and increased WBC values in the urogenital tract (n = 4) and abnormal WBC in hole blood and normal urogenital parameters (n = 4).

Parameters of peripheral blood in this group were compared with the group with normal values. The most numerous group had abnormal parameters both in blood and in cervix or vagina.