EFFECT OF HERPESVIRUS PERSISTENCE ON THE FORMATION OF A SPECIFIC IMMUNE RESPONSE IN CHILDREN

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According to the WHO forecasts, the diseases caused by herpesvirus infections (HVI) in the near future are defined as "the global problem of mankind". A feature of HVI is that the immune system responds to the extracellular location of free virus particles or antigenic determinants, rather than to latent viruses in nerve ganglia, macrophages, lymphocytes, etc.: immune system reactions are not observed. HVI can provoke functional disorders in the cells of the immune system: macrophages, T-lymphocytes, violation of the blast-cell transformation of lymphocytes. Hence, apparently, there is a clinically sluggish state with seemingly normal (numerically) indices of cellular immunity, which requires immune therapy to activate the function of immunocompetent cells [1].

The best way to reduce the incidence of vaccine-preventable infections is to create a population of highly immune individuals. This is achieved through the implementation of immunization programs. The main focus of most immunization programs are young children, for whom WHO offers routine immunization against diphtheria, tetanus, whooping cough, measles, epidparotitis, rubella, and poliomyelitis in developed countries.

To date, the reasons for the different immune response of people to antigens (natural infections and vaccines) have been little studied. Presumably, the strength of the immune response depends on the individual set of genes of the main histocompatibility complex (MHC), which products are responsible for the recognition and presentation of antigens. Alleles of genes that provide proper presentation and recognition of the most important antigenic structures were selected during evolution.

The state of individual immunoreactivity is determined by the genotype of the organism, therefore there are always highly reactive individuals (HRI) ≈ 20 %, moderately reacting (MRI) ≈ 50–70 %, areactive (not responding to the antigen) (ARI) ≈ 10 % in the population. The presence of immunodeficiency prevents or makes impossible the formation of postvaccinal immunity.

According to [3, p.207], the problem of herpesvirus infections is most acute in pediatrics, which is associated with a poor knowledge of epidemiology, immunopathogenesis, clinical manifestations, therapy and, most importantly, prevention of exacerbations of HVI. The frequency of occurrence is now more than 40%. The most, perhaps, unpleasant is the increased number of relapsing forms in early school age (6-7 years). Recently there has been an increase in the number of people with secondary immunodeficiency, whose active immunization is ineffective [4, p.45]. Most herpesviruses belong to opportunistic infections: they clinically manifest themselves in conditions of immunodeficiency (ID). On the other hand, the manifestation of herpetic infection (HI) has in turn an immunosuppressive effect, which creates the prerequisites for the subsequent reactivation of the latent infection and its possible expansion. The course of HI is accompanied by significant disturbances in the regulation of the immune response due to the inferiority of the interleukin (IL) system. In particular, the ability of infected immunocompetent cells to synthesize IL is impaired, and the response of target cells to them is changing.

The purpose of this study was to establish the relationship between the formation of specific post-vaccination immunity and persistence of various members of the Herpesviridae family in children of preschool age.

Material & methods

145 children aged 1 to 7 years old who received the first CPC vaccine according to the vaccination schedule of Ukraine were examined. Class G antibodies (Ab) to measles, rubella and parotitis viruses were determined by ELISA. The threshold concentration of Ab was calculated in IU / ml according to the instructions for the test systems that were used. Antibodies of the IgG class for the measles virus were determined using an enzyme immunoassay test system manufactured by IBL international GMBH - Meales virus IgG ELISA (Germany). Antibody concentrations were calculated in international units per ml (mIU / mLA threshold concentration was considered 200 mIU / mL. Detection of antibodies at a concentration of 200-300 mIU / mL was considered as evidence of conditional protection, detection of antibodies at a concentration of more than 300 mIU / mL as evidence of protection against measles. Rubella IgG antibodies were determined using the Rubella IgG ELISA test system from Xema Co.Ltd. (Kiev, Ukraine). Antibody concentrations were calculated in international units per ml (IU / ml). A threshold concentration of 15 IU / ml was considered. Detection of antibodies at a concentration of 15-25 IU / ml was considered as evidence of conditional protection, detection of antibodies at a concentration of 25-73.5 IU / ml as evidence of protection against rubella (projective level of antibodies). Antibodies IgG antibodies were determined using the enzyme immunoassay test system manufactured by R-Biopharm AC (Germany) - RADASCREEN® Mumps Virus IgG (K5521). Antibody concentrations were calculated in international units per ml (IU / mL). The threshold concentration was considered to be 14.0 IU / mL. Detection of antibodies at a concentration of 14.0-24.0 IU / mL was considered as evidence of conditional protection, detection of antibodies at a concentration of more than 24.0 IU / mL as evidence of protection against mumps. Determination of the antigens (Ag) of the Herpesviridae family was performed by immunofluorescence method using specific monoclonal mouse antibodies of Santa Cruz Biotechnologu, Inc. (USA), whose supplier was Bio Test Med LLC (Kiev, Ukraine):

– Recombinant Cytomegalo Virus Mosaic («SANTA CRUZ», USA);

– Recombinant Epstein-Barr Virus (HHV-4) Mosaic EBNA1 («SANTA CRUZ», USA);

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– Recombinant Herpes Simplex Virus-1 gG («SANTA CRUZ», USA);

– Recombinant Herpes Simplex Virus-2 gG («SANTA CRUZ», USA);

– Recombinant Varicella Zoster Virus gE («SANTA CRUZ», USA);

– Recombinant Herpes Simplex Virus-6 Mosaic («SANTA CRUZ», USA).

With the observance of measures from the patient, blood was taken. By the standard method, leukocyte mass was obtained. On degreased slides prepared thin smears of cell mass. After drying, the swabs were fixed in methanol for 15 min, then the working dilution of specific serum was applied to the smears for 15 min, and after washing with distilled water, anti-species immunoglobulins were labeled, FTIC-labeled and smears were placed for 25 min in a thermostat at (37 ± 0.5) ° C. After this time, the smears were washed with distilled water, dried with filter paper and viewed in a Zeiss Primo Star luminescence microscope using an immersion system and non-fluorescent oil. Antigen-containing cells were identified by the nature and intensity of cell luminescence.

Statistical processing of the results was performed using the statistical package of the computer program Microsoft Excel Windows XP.

Results & discussion

When analyzing the results of the research conducted, the following was established.

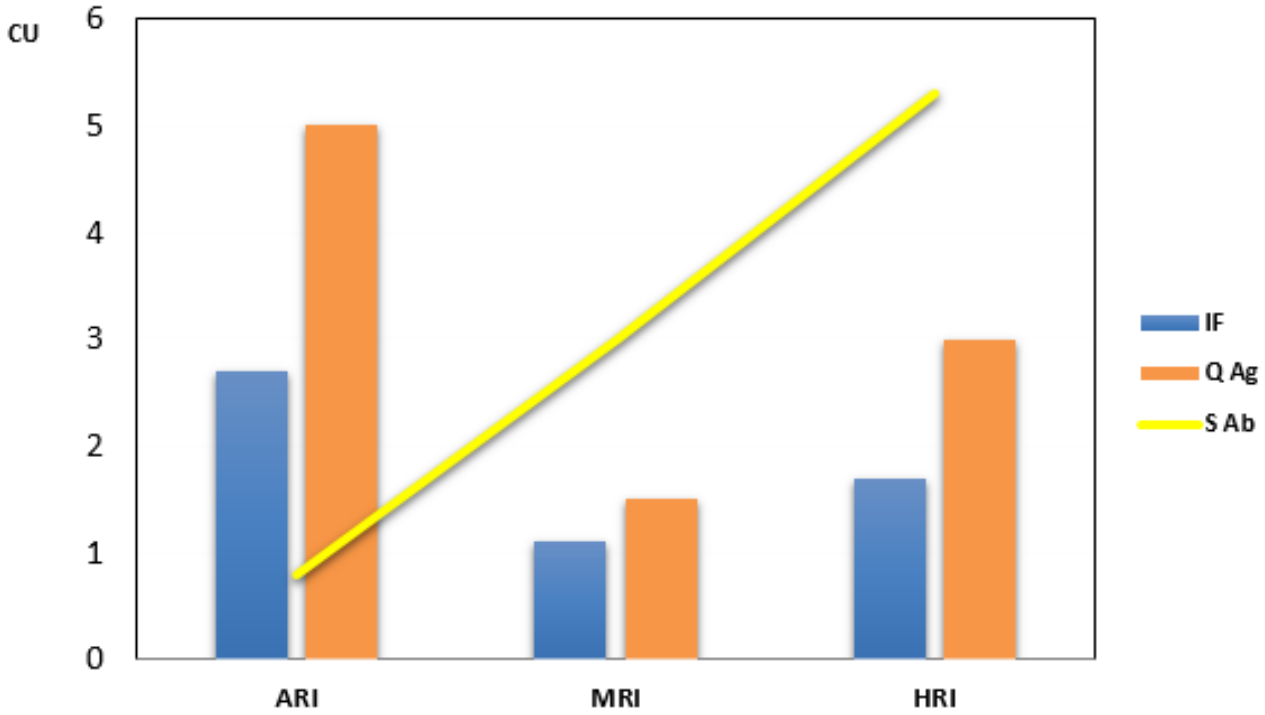

Figure 1. The relationship of a specific immune response with the persistence of herpes viruses in the body of children under 7 years of age

When analyzing the relationship between the persistence of herpesviruses and the imbalance of a specific immune response, it was noted that the absence of vaccinal immunity on the MMR correlates with the high viral load and the presence in the body of the child more than 3 representatives of herpes viruses. With hyperreactivity of a specific immune response, a direct relationship is observed with the detection of a combination of VEB + CMV + HHV6. It is precisely this combination of these heresviruses that has always been detected in children with superhigh titers of Ab in the MMR vaccine. (Fig.1).

Our studies showed that, on average, 17 ± 3.4% of children immunized against measles and rubella were vaccinated against measles and rubella, and 1.7 times more did not respond with the production of specific Ab in the mumps component of the vaccine. Especially it is necessary to pay attention to the fact that more than half of the children after the first vaccination have hypertensions Ab to rubella and, almost every third child, to measles and mumps. Especially the percentage of hyperreactivity increases in children 5-6 years, i.e. by the time the MMR is revaccinated, according to the vaccination schedule. At the time of vaccination, the child must be healthy. Ideally and even more so when there are doubts, on the eve of the vaccination, a general blood test should be done. As our studies show, it is also necessary to conduct a study to identify the persistence of herpes simplex viruses in the child's blood cells. The depth of functional immunodeficiency is caused not only by the magnitude of the viral load, but also by the combination of herpetic infections (Fig.2).

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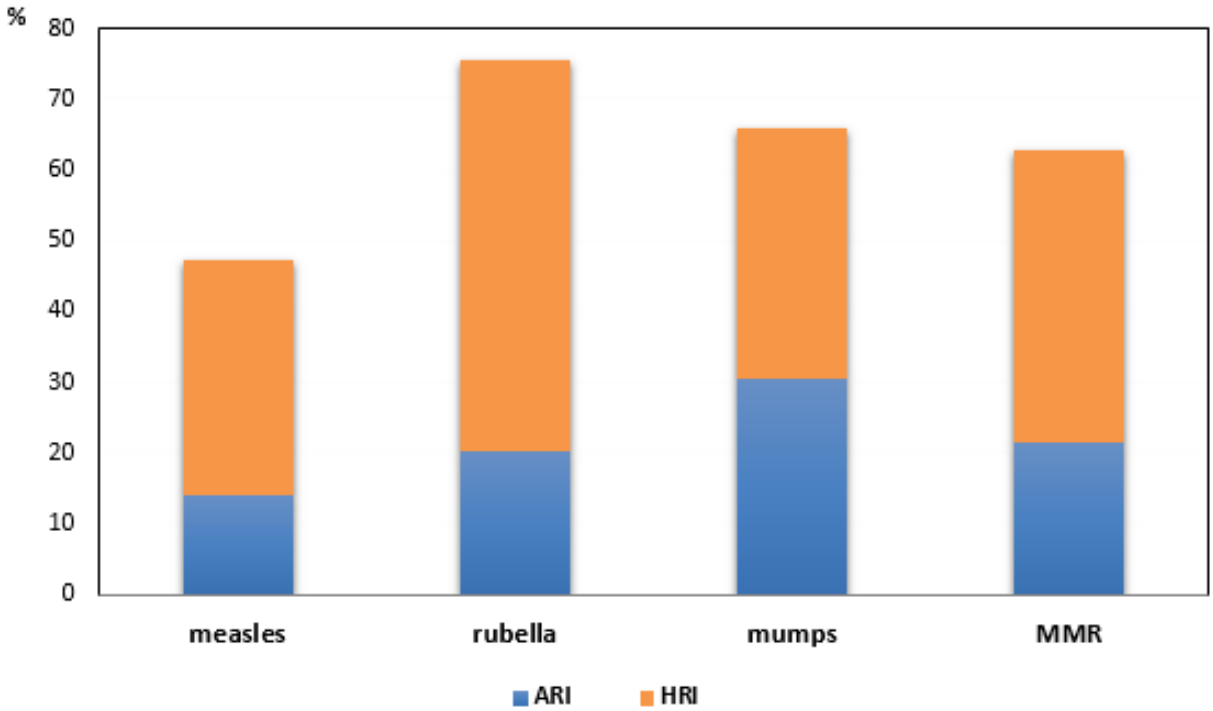

Figure 2. Imbalance of a specific immune response in the persistence of herpes viruses in the body of children under 7 years of age

Excess immunization is unjustified in terms of medical ethics and economy. Due to the constant circulation of some pathogens, there is a natural immunization of people without vaccination. Some of them have a high initial level of antibodies and do not need to be vaccinated. Other individuals genetically produce high antibody titers during vaccination and do not require revaccination. It should be borne in mind that with intensive antibody formation, revaccination is unnecessary and undesirable. A high level of antecedent antibodies can inactivate the injected antigen, partially reduce the intensity of immunity as a result of neutralizing antigens with available antibodies. Therefore, it is desirable, but impossible for everyone today, to have a pre-vaccination screening - a serological examination of persons subject to vaccination for the presence of immunity to the infection. Typically, the goal of pre-vaccination screening is to identify non-immune (seronegative for the causative agent of a specific infection) individuals. In rare cases, pre-vaccination screening is conducted because of the undesirability of exposing immune individuals to additional allergies associated with vaccination. This makes it possible to determine the need for immunization, to cancel further vaccination in persons with strained immunity or, conversely, to take measures to strengthen the immune response in the vaccinated person.

Advances in the fight against measles, rubella and parotitis depend primarily on the correct strategy and tactics of vaccination, the level of vaccination coverage and the quality of the vaccine preparation. At the same time, global synchronicity and coherence of anti-epidemic measures in all world regions is of great importance. However, in many countries of the world, the importance of combating these infections is still underestimated, which creates conditions for the preservation of foci of diseases in certain territories and their export to other countries.

Conclusion

First of all, the principles of individual vaccination should be extended to risk groups, which, we believe, should include children with persistent herpes virus infection, which causes both the development of secondary immunodeficiency and allergic organism. In order to make the best choice of vaccine and to give patients informed recommendations, it is necessary to have knowledge of the reactogenicity and immunogenicity of the drugs, as well as have up-to-date information on changes in vaccination strategies and tactics, on the individual status of children, on the possible long-term effects of the use of an immunobiological preparation or the consequences of not using it.

Total pre-vaccination and post-vaccination screening is not carried out in any country of the world, although the universal success of global vaccination is still not coming.

Among the reasons for the deterioration of the epidemiological situation in our country, along with incomplete vaccination coverage, are the following: insufficient effectiveness of the vaccines used, violation of the rules for handling vaccines and depletion of vaccination immunity, possibly caused by the persistence of herpes viruses. Due to these reasons, even despite the massive vaccinations, one part of the children does not have protective immunity and needs additional vaccination, and the other has hypertitres of specific antibodies and does not need revaccination. Identification of such persons can be made only by serological examination. Complete and reliable information not only about the incidence, but also about the state of specific population immunity in different

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age groups, will allow to predict the epidemic situation and to conduct vaccination measures in person.

References:

1. Communicable diseases 2000. - Geneva: WHO/CDS. – 2016
2. Decade of Vaccines — Global Vaccine Action Plan 2011-2020/ http://www.who.int/immunization/global_vaccine_action_plan/DoV_GVAP_2012_2020/en/
3. Cherevko N.A. Popova I.S. Klimov V.V., etc. Features of change of population immunity of the population against herpes-infection / Russian journal of Allergology. 2010. N. 1 (1). P. 207–208.
4. Klimova R.R. Frequency of detection of markers of herpesvirus infections in frequently ill children with acute respiratory infection // Pediatrija. 2014. № 93 (1). P. 44—49.
5. Nagoyev B.S., Kambachokova Z.A. Condition of cellular immunity at patients with herpes infection / Messenger of new medical technologies 2011. N. 3 (18). P. 325–327.
6. Toptygina A.P., Asiatseva V.V., Savkin I.A.et al. Prediction of a specific humoral immune response based on the initial parameters of the immune status of children vaccinated against measles, rubella and mumps // Immunology. 2015. Vol. 36 (1). P. 34-41.

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Introduction. The best way to reduce the incidence of vaccine-preventable infections is to create a population of highly immune individuals. This is achieved through the implementation of immunization programs. The main focus of most immunization programs are young children, for whom WHO offers routine immunization against diphtheria, tetanus, whooping cough, measles, epidparotitis, rubella, and poliomyelitis in developed countries. According to the WHO forecasts, the diseases caused by herpesvirus infections (HVI) in the near future are defined as "the global problem of mankind". A feature of HVI is that the immune system responds to the extracellular location of free virus particles or antigenic determinants, rather than to latent viruses in nerve ganglia, macrophages, lymphocytes, etc.: immune system reactions are not observed. HVI can provoke functional disorders in the cells of the immune system: macrophages, T-lymphocytes, violation of the blast-cell transformation of lymphocytes. Hence, apparently, there is a clinically sluggish state with seemingly normal (numerically) indices of cellular immunity, which requires immune therapy to activate the function of immunocompetent cells. According to, the problem of herpesvirus infections is most acute in pediatrics, which is associated with a poor knowledge of epidemiology, immunopathogenesis, clinical manifestations, therapy and, most importantly, prevention of exacerbations of HVI. The frequency of occurrence is now more than 40%. The most, perhaps, unpleasant is the increased number of relapsing forms in early school age (6-7 years). Recently there has been an increase in the number of people with secondary immunodeficiency, whose active immunization is ineffective. The purpose of the study. We studied the relationship between the formation of specific postvaccinal immunity and the persistence of various representatives of the Herpesviridae family in children under 7 years old who received the first vaccine of the PDA according to the vaccination schedule of Ukraine. **Material and methods.** 145 children aged 1 to 7 years were examined. Antibodies (Ab) of class G to measles, rubella and mumps viruses were determined by the ELISA method. The threshold concentration of Ab was calculated in IU/ml according to the instructions for the test systems that were used. Immunofluorescence and PCR were used to detect viral antigens (Ag) and DNA, respectively. **Results and discussion**. When analyzing the relationship between the persistence of herpesviruses and the imbalance of a specific immune response, it was noted that the absence of vaccinal immunity on the MMR correlates with the high viral load and the presence in the body of the child more than 3 representatives of herpes viruses. With hyperreactivity of a specific immune response, a direct relationship is observed with the detection of a combination of VEB + CMV + HHV6. It is precisely this combination of these heresviruses that has always been detected in children with superhigh titers of Ab in the MMR vaccine. Our studies showed that, on average, 17 ± 3.4% of children immunized against measles and rubella were vaccinated against measles and rubella, and 1.7 times more did not respond with the production of specific Ab in the mumps component of the vaccine. Especially it is necessary to pay attention to the fact that more than half of the children after the first vaccination have hypertensions Ab to rubella and, almost every third child, to measles and mumps. Especially the percentage of hyperreactivity increases in children 5-6 years, i.e. by the time the MMR is revaccinated, according to the vaccination schedule. At the time of vaccination, the child must be healthy. Ideally, and even more so when there are doubts, on the eve of the vaccination, a general blood test should be done. As our studies show, it is also necessary to conduct a study to identify the persistence of herpes simplex viruses in the child's blood cells. The depth of functional immunodeficiency is caused not only by the magnitude of the viral load, but also by the combination of herpetic infections. Excess immunization is unjustified in terms of medical ethics and economy. Some of them have a high initial level of antibodies and do not need to be vaccinated. Other individuals genetically produce high antibody titers during vaccination and do not require revaccination. It should be borne in mind that with intensive antibody formation, revaccination is unnecessary and undesirable. **Conclusion.** Therefore, it is desirable, but impossible for everyone today, to have a pre-vaccination screening - a serological examination of person’s subject to vaccination for the presence of immunity to the infection. Typically, the goal of pre-vaccination screening is to identify non-immune (seronegative for the causative agent of a specific infection) individuals. In rare cases, pre-vaccination

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screening is conducted because of the undesirability of exposing immune individuals to additional allergies associated with vaccination. This makes it possible to determine the need for immunization, to cancel further vaccination in persons with strained immunity or, conversely, to take measures to strengthen the immune response in the vaccinated person. First of all, the principles of individual vaccination should be extended to risk groups, which we believe should be attributed to children with persistent herpesvirus infection, which causes both the development of secondary immunodeficiency and allergic organism.
Key Words: vaccine-preventable infections, herpesvirus infections, vaccination

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