During the last two years of observation the molecular-genetic research allowed to reveal circulation of the two Far Eastern *CA10* genetic lines of different origins and identify the time to their MRCA.

3.40 doi: 10.15789/2220-7619-2018-4-3.40

DISTRIBUTION OF ROTAVIRUS G-, P-, I-, AND E-GENOTYPES IN NIZHNY NOVGOROD, RUSSIA

T.A. Sashina[1], O.V. Morozova[1,2], N.V. Epifanova[1], T.A. Migunova[2], N.A. Polyakov[2], N.A. Novikova[1,2]

[1]I.N. Blokhina Research Institute for Epidemiology and Microbiology, Nizhny Novgorod, Russia, [2]Lobachevsky State University, Nizhny Novgorod, Russia

Rotavirus infection is an important health problem all over the world. In Russia, under the conditions of the beginning of vaccination against this infection, knowledge about its pathogen is limited by the characteristic with the binary classification (G[P]-genotypes), based on the properties of the VP4 and VP7 genes encoding the rotavirus outer capsid proteins. Information about the other gene segments genotypes, as well as unusual and reassortant strains is not sufficient. The aim of this study was to determine the I (VP6) and E (NSP4) genotypes of rotaviruses detected in Nizhny Novgorod using the multiplex PCR method.

We used 55 rotavirus-positive fecal samples from children hospitalized with acute intestinal infection from January to May 2018. RNA of rotaviruses was extracted using "RIBO-prep" reagent kit (AmpliSens, Russia). RT-PCR was carried out with reagents manufactured by "Sileks" (Germany). G- and P-genotypes of rotaviruses were determined using previously published primers. To identify I- and E genotypes in multiplex PCR, fragments of 195 bp (I3), 273 bp (I1), 368 bp (I2) and 233 bp (E3), 305 bp (E2), 443 bp (E1), respectively, were amplified and detected by agarose gel electrophoresis.

I- and E-genotypes were determined in 51 samples (92.8%). In one sample only E-genotype (1.8%) was revealed, and in three — only I-genotype (5.4%). Mostly, the genotypes were detected in combination I1-E1 (52.7%). The set of I2-E2 was found in 30.9% of cases. In addition, the genotype I1-E2 (5.6%) was identified in three samples, I2-E1 and I3-E3 (3.6% together) were shown to be sporadic. The following combinations of G-, P-, I-, and E-genotypes were determined: G1-P[8]-I1-E1 (9.1%), G4-P[8]-I1-E1 (7.3%), G9-P[8]-I1-E1 (32.7%), G4-P[8]-I1-E2 (5.5%), G3-P[x]-I2-E2 (1.8%), G2-P[4]-I2-E2 (29.1%), G2-P[4]-I2-E1 (1.8%), G2-P[4]-I2-Ex (3.6%), G9-P[8]-I1-Ex (1.8%), Gx-P[8]-I1-E1 (5.5%), and Gx-P[x]-I3-E3 (1.8%).

Thus, the new method to identify the I- and E-genotypes was tested and their distribution was determined. Various combinations of G-, P-, I-, and E-genotypes of rotaviruses have been shown. The genotype G9-P[8]-I1-E1 was predominant (32.7%). The G4-P[8]-I1-E2, G3-P[x]-I2-E2, G2-P[4]-I2-E1 strains had probably a reassortant origin.

The study was funded by RFBR (research project No. 18-34-00586).

3.41 doi: 10.15789/2220-7619-2018-4-3.41

DIAGNOSIS OF CYTOMEGALOVIRUS AND PARVOVIRUS B19 INFECTIONS IN SPECIAL GROUPS OF PATIENTS

V.M. Semenov, T.I. Dmitrachenko, V.U. Harbachou, A.V. Rednenko

Vitebsk State Medical University, Vitebsk, Republic of Belarus

Some researchers described the reactivation of cytomegalovirus infection in immunocompetent patients with sepsis, burns, blood transfusions, massive surgical interventions, prolonged mechanical ventilation, use of steroids and vasopressors. In addition to herpesviruses, the reactivation of other latent viruses, in particular, parvovirus B19 (B19V), can also occur with developing immunodeficiency phenomena. With the existing concomitant pathology, these viruses significantly burdens the condition of patients.

For this reason the need for a qualitative and timely diagnosis of viral infections is increasing. PCR assay which capable of detecting even a few molecules of DNA is a progressive diagnostic method due to its high sensitivity. In this regard, quantitative detection of viral DNA can serve as a reliable criteria for significant activity of the pathogen, proving its etiological role in the development of a clinical syndromes.

The aim of the study was to create a test systems for quantitative DNA detection of CMV and B19V with hybridization-fluorescent detection of amplification products in the "real time" mode. It will help to establish the frequency of reactivation of latent viral DNA in critical condition and subsequently determine its effect on the course of the pathological process.

As a result of the studies for the first time in the Republic of Belarus a test systems for the qualitative and quantitative detection of CMV and B19V DNA by the real-time PCR method was created and registered by the Ministry of Health. The main characteristics of the developed test systems showed high values of analytical sensitivity (≥ 2 copies per run of 500 ME/ml), analytical and diagnostic specificity (100%), linear range (> 8 logarithms).

The created test systems, in addition to its use as a diagnostic tool, also can be used as a prognostic marker of infection, as a therapeutic marker for monitoring the success of antiviral therapy as well as for assessing the contagious nature of biological fluids. Thus, during the conducted studies using the test system, reactivation of CMV was detected in 28.6% (6 of 21) of patients in a critical condition with a viral load of 10 to 111 copies/ml. Also, a strong correlation between reactivation of CMV and established diagnosis of sepsis was found ($r = 0.73$). Reactivation of B19V was not detected in any of the 15 patients, which is inconsistent with the existing literature data and requires further researches.

3.42 doi: 10.15789/2220-7619-2018-4-3.42

IMPROVEMENT OF TECHNOLOGY OF PRODUCTION OF HERPETIC VACCINE, CULTURAL, INACTIVE

G.S. Shitikova, E.P. Turova

FGUP SPbNIIVS FMBA Russia, St. Petersburg, Russia

To improve methods of production and control of the vaccine, with the aim of developing a new innovative form of herpetic vaccine.

Vaccine strains of herpes simplex virus (HSV) type I (strain "US") and type II (strain "VN") are used as a seed material for preparing of herpesvirus vaccine. The monolayer cell culture (CC) of the primary fibroblasts of chick embryos (FECH) and the diploid cells of the human lung embryo (FLECH) were used for preparing of vaccine. Harvest virus of HSV-I and II types are collected in semi-finished products, which after freezing and thawing are inactivated with formalin. In a comparative plan, the semi-finished products accumulated on different cellular substrates are monitored, in accordance with the production schedule and the current regulatory documents. Semi-finished products are controlled for infectious activity, safety, toxicity, and absence of extraneous contamination. Control of specific activity is carried out in experiments on white rats.

It was necessary to use a high concentration of plant vaccine strains of HSV-I and II types when creating an innovative form of herpetic vaccine. We received a high yield of the virus when growing viruses on a cell culture of human origin, in particular, in the CC FLECH. It was found that the reproductive activity of vaccine strains on the FLECH was 6.0–6.5 lg TCD 50/ml. It was higher in 10–100 times than the activity of their reproduction on the FECH QC for HSV strains I and II type, respectively. The specific activity of semi-prepared foods prepared in different cell cultures was studied in animals. It was found that the specific activity of semifinished products manufactured on the CC FLECH exceeded by 10 times the activity of the semifinished products obtained at the FECH QC, which was determined by the index of neutralization of the sera of immune animals.

Conditions for increasing the reproductive activity of HSV seed strains have been developed, the concentration of viral antigens in the vaccine has been increased 10–100 times, using a substrate of diploid cell culture of human origin, compared with CC FECH. Methods for the production of herpetic vaccine have been improved, and the basis for the creation of a new innovative form of herpetic vaccine has been developed.

3.43 doi: 10.15789/2220-7619-2018-4-3.43

IMMUNIZATION WITH UNIVERSAL INFLUENZA VACCINE ENHANCES IMMUNE RESPONSE TO SUBSEQUENT INFECTION

M.A. Shuklina, L.A. Stepanova, I.G. Vidyaeva, A.V. Korotkov, E.I. Eletskaya, L.M. Tsybalova

Smorodintsev Research Institute of Influenza, St. Petersburg, Russia

This study evaluated the cellular and humoral responses, relative to conserved viral M2 and HA antigens, of previously immunized mice to sublethal influenza infection. We developed an experimental, recombinant protein universal vaccine Flg-HA2-4M2e features a hemagglutinin second subunit (aa76–130) consensus fragment of influenza A viruses belonging to phylogenic group 2 (HA2) joined with 4 tandem copies of M2e (viral M2 protein ectodomain); those fragments were sequentially linked to the C-terminus of flagellin. BALB/c mice were immunized intranasally 3 times (2 wk intervals, 10 μg/0.02ml); controls were administrated PBS, as above. Two weeks after final immunization, immunized and control mice were challenged with a sublethal dose (100MID) of influenza A/Aichi/2/68 (H3N2). Post-vaccination humoral immune response was characterized by high levels (serum, BAL) of anti-M2e IgG and IgA. One month post challenge, anti-M2e IgG levels in immunized mice were elevated 1.5 fold. In controls, infection did not lead to anti-M2e IgG formation in serum. Anti-M2e IgA in BAL was increased 3.5 fold in immunized mice and only 1.7 fold in controls. A significant rise in IgG titers against A/H3N2 virus in immunized mice (5.6 fold) compared to controls (2.5 fold) was noted. In lung, the post-vaccination response was characterized by the formation of M2e- and HA2 specific T-cells ($CD4^+$, single (TNF^+) and double (TNF^+IL2^+) producing effector memory cells — Tem). One month after challenge, TNF^+ and TNF^+IL2^+ M2e-specific T-em levels increased almost 10-fold. Double producers (IFN^+TNF^+) and triple producers ($IFN^+TNF^+IL2^+$) were also detected. The pool of HA2-specific double producing Tem ($TNF^+IL\text{-}2^+$) increased significantly (~4x), and TNF^+ mono and $IFN^+TNF^+IL2^+$ triple producers appeared. In control mice, infection resulted in the formation of fewer specific Tem cells. The results show that sublethal infection in mice pre-immunized with Flg-HA2–4M2e: enhanced Ag-specific local and systemic humoral responses; increased Ag-specific Tem lung populations; and led to the appearance of new cytokine secreting effector T memory cells.

Supported by: Russian Science Foundation (Project No. 15-14-00043-П).

3.44 doi: 10.15789/2220-7619-2018-4-3.44

FEATURES OF POPULATION IMMUNITY AGAINST MEASLES AND RUBELLA VIRUSES. WHY DO ADULTS SUFFER?

M.A. Smerdova[1], A.P. Toptygina[1,2], M.A. Naumova[1], N.P. Vladimirova[3], T.A. Mamaeva[1]

[1]G.N. Gabrichevsky Research Institute for Epidemiology and Microbiology, Moscow, Russia; [2]Lomonosov Moscow State University, Moscow, Russia; [3]Centre for Hygiene and Epidemiology in Moscow, Moscow, Russia

The main factor in the immunity of people to measles and rubella viruses is the presence in the blood of the protective level of specific antibodies. These antibodies appear both after disease, and after vaccination. The level of antibodies is maintained for many years by long-lived plasmacytes and memory B-cells. Repeated contact with the virus leads to the boost — an increase in the level of specific IgG. However, in the conditions of intensive vaccination of the population, the circulation of the wild virus is reduced and the probability of natural boosting vaccinated people with wild strains of viruses is reduced. According to the Russian vaccination calendar, vaccinations against measles and rubella viruses are given to children at 1 year and 6 years of age. At the same time, among the measles cases, a group of young adults 20–40 years old is singled out, which raises the question of the duration of postvaccinal immunity. Using "Vector Best" kits, the study of the anti-measles and anti-rubella immunity was conducted of age groups: up to 1 year, 1–2 years, 3–6 years, 7–14 years, 15–17 years, 18–30 years, 31–40 years, 41–50 years and 51–60 years on the territory of Moscow and the Moscow region for 2013 (the territory with an unfavorable epidemic situation). The serum from 654 randomly selected healthy individuals and 646 patients from the same region with a serologically confirmed measles infection were examined. A gradual increase in the percentage of people with protective levels of antibodies to rubella and measles viruses was found, reaching 81.3% for measles and more than 90% for rubella at the age of 7–14 years. At the same time, the percentage of those protected against rubella remained at an older age. While the most pronounced increase in the seronegative persons to measles virus (40% or more) in the 18 to 30-year-old age group was found, but in groups older than 40 years, the immunity reached 85–95%. A strong negative correlation was found between the incidence of measles and the level of tension of the population's anti-measles immunity ($r = -0.76$). Thus, an increase in the number of cases of sickness to 28% at the age of 18–30years and a decrease to 2.9% in 51–60 years was provided by a decrease (up to 55%) and an increase (up to 95%) of persons with protective immunity, respectively.