NONPROFIT PARTNERSHIP "ORCHEMED": FROM BASIC RESEARCH TO NOVEL DRUGS

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In 2006, the BioIndustry Initiative (BII) of the U.S. Department of State and the ISTC started to fund a project entitled "Identification, addition and realization of the nascent value of novel compounds as drug candidates through preclinical safety and efficacy testing and technology transfer" (ISTC Project 3283), to be conducted by Noncommercial Partnership "Orchemed"[1], a partnership among several academic chemistry Institutes of the Russian Academy of Sciences. The main goal of the ISTC Project 3283 is the development of innovative activity of the Russian academic institutes working in the field of chemistry and drug discovery. It was also planned to perform preclinical trials of the most promising lead-compounds, developed at these institutes, using the reconstructed technical and animal facilities of JSC "TRUST," founded by NP "Orchemed."

In the framework of this project, the "Orchemed" experts select promising hit-compounds and recommend them for further preclinical studies at JSC "TRUST." In addition to the comparative evaluation of candidates' efficacy *in vitro* and *in vivo*, available results of their assessment in diseases models, technological effectiveness of compound syntheses and novelty, the priority selection criteria include market research results obtained for the international and domestic pharmaceutical markets (demand for products from the relevant pharmaceutical groups). To date, several valuable preclinical candidates were selected by the "Orchemed" expert team. In particular, RU32 and a series of related compounds developed at IPAC RAS represent conceptually new group of cognitive enhancers of new generation that are capable not only improve memory storing abilities, but also restore completely lost fragments of memory, so-called effect of "total recall". Compound BG-122 (INEOS) is demonstrating significant antiviral activity against human influenza viruses and avian influenza strains. Compound LS-17 (IOS UrBRAS) possesses significant antiaggregant and hypotensive activity. The novel developments include advanced nanoparticle-based drug delivery systems, novel antiinflammatory, anti-HIV, antitumor and cardiovascular drugs, etc. The current research portfolio of NP «Orchemed» includes more than 50 projects in actual pharmaceutical areas.

Today, NP «Orchemed» is a leading Russian academic group in the field of drug discovery and development, and includes 11 institutes. As an independent expert group NP "Orchemed" took part in preparation of a novel Strategy of Development of Pharmaceutical Industry of Russian Federation in 2008-2020[2]. Research activity of NP «Orchemed» is supported by foreign and Russian governmental and academic organizations.

DEVELOPMENT OF A NEW ORAL RABIES VACCINE RABIVAC-O/333

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The new oral rabies vaccine "Rabivac-O/333" was developed at the Pokrov Plant of Biologics in Russia during the implementation of ISTC #2090/BTEP #50 Project. The vaccine is fishmeal bait containing a plastic blister filled with 2.0 ml of ERA G333 rabies virus strain. The ERA G333 virus was created at CDC and kindly provided to us within the collaborative ISTC Project. The main purpose of our work was to study biological properties of ERA G333 rabies virus strain, such as safety for target and non-target animals, immunogenicity, and virus propagation in BSR and BHK-21 cells. Additionally, our goal is to create a bait which is safe and attractive for wild carnivores. Our study showed that this strain is avirulent for 11 species of target and laboratory species even if inoculated intracerebrally and caused rabies only in suckling mice. ERA G333 rabies virus strain is highly immunogenic for red foxes and raccoon dogs inoculated orally in laboratory experiments and field trials. A field trial of bait acceptance by wild animals showed high attractiveness for wild carnivores. The consumption of baits was registered at control points every day by the presence of baits, blisters, and traces of animals. About 30% of baits were consumed at the first day after baiting and by the 4th day 99.3% were eaten.

Considering the vaccine safety and appropriate immunogenicity for wild carnivores, as well as the results of a study of bait consumption by wild animals, it can be concluded that oral rabies vaccine "Rabivac-O/333" is safe, immunogenic, and attractive for target species and suitable for wildlife oral immunization.

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THE FIRST INTERNATIONALWORKSHOP ON PRACTICAL FLOW CYTOMETRY

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The 1st International Workshop on Practical Cytometry was held at the Russian State Hematology Center in Moscow, Russia on 22-27 May 2009. The workshop was jointly organized by Drs. Natasha Barteneva (Harvard University Medical School, USA) and Ivan A. Vorobjev (State Hematology Center, Moscow, Russia). Eighty-two students from Russia, Belarus, Ukraine, Kazakhstan, Kirgizstan, Moldova and Switzerland participated in a hands-on workshop presenting the latest developments in research and clinical flow cytometry.

A distinguished group of twenty four Russian and US faculty members presented seminars and conducted wet laboratories on a wide variety of flow cytometry technologies and applications, including Dorothy Lewis (Baylor College of Medicine, USA), Albert and Vera Donnenberg (University of Pittsburgh School of Medicine, USA), Nina Drize (State Hematology Center, Moscow), Joanne Lannigan (University of Virginia, USA), Irina Lyadova (Central Institute for Tuberculosis, Moscow), Michael Andreeff (MD Anderson Cancer Center, USA), Vincent Shankey (Beckman-Coulter), Vladislav Verkhusha (Albert Einstein School of Medicine, USA) and Sergei Lukyanov (Institute for Bioorganic Chemistry, Moscow), among others.

Seminar and wet lab subjects included basic flow cytometry theory and practice, fluorescence technology, fluorescent immunolabeling for basic research and clinical analysis, apoptosis, cytotoxicity, rare cell detection, image cytometry, fluorescent protein analysis, cytokine labeling, cell sorting and intracellular antigen analysis. Dr. Vorobjev's laboratory at the State Hematology Center provided BD Biosciences cytometry instrumentation to support the wet labs, and instrument manufacturers Beckman-Coulter and Accuri loaned additional state-of-the-art Gallios and Accuri C6 cytometers to support the workshop. Students had one-on-one access to all faculty throughout the program, to provide advice on their specific projects and applications. Plans are already being made to conduct this workshop again in 2010 and beyond.

This meeting received generous support from the International Science and Technology Center (ISTC), Russian Foundation for Basic Research (RFBR), The Russian State Hematology Center, the Coulter Foundation and the International Society for Advancement of Cytometry (ISAC). Corporate support was provided by Accuri Cytometers, Beckman-Coulter, Bioline, Compucyte Corporation, Cytek Development, De Novo Software, Invitrogen Life Technologies and Miltenyi Biotec.

NOVEL CONJUGATE OF MOXIFLOXACIN AND CARBOXYMETHYLATED GLUCAN WITH ENHANCED ACTIVITY AGAINST MYCOBACTERIUM TUBERCULOSIS

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Mycobacterium tuberculosis is an intracellular pathogen that persists within macrophages of the human host. One approach to improving the treatment of tuberculosis (TB) is the targeted delivery of antibiotics to macrophages using ligands to macrophage receptors. The moxifloxacin-conjugated dansylated carboxymethylglucan (M-DCMG) conjugate was prepared by chemically linking dansylcadaverine (D) and moxifloxacin (M) to carboxymethylglucan (CMG), a known ligand of macrophage scavenger receptors. The targeted delivery to macrophages and the antituberculosis activity of the conjugate M-DCMG were studied in vitro and in vivo. Using fluorescence microscopy, fluorimetry, and the J774 macrophage cell line, M-DCMG was shown to accumulate in macrophages through scavenger receptors in a dose-dependent (1 to 50 μg/ml) manner. After intravenous administration of M-DCMG into C57BL/6 mice, the fluorescent conjugate was concentrated in the macrophages of the lungs and spleen. Analyses of the pharmacokinetics of the conjugate demonstrated that M-DCMG was more rapidly accumulated and more persistent in tissues than free moxifloxacin. Importantly, therapeutic studies of mycobacterial growth in C57BL/6 mice showed that the M-DCMG conjugate was significantly more potent than free moxifloxacin.

Many therapy-related problems could be attenuated or potentially eliminated through selective delivery of anti-TB drugs into infected macrophages, the primary site of infection. Unlike many other cell types, macrophages are known to express high levels of specific receptors on their plasma membrane that bind and internalize their specific target ligands through a variety of uptake mechanisms, such as specific polysaccharide receptors and macrophage scavenger receptors that bind anionic macromolecules and use phagocytosis for ligand uptake. Chemical labeling of glucans with carboxy or sulfate groups can lead to their selective accumulation by tissue macrophages via scavenger receptor-mediated uptake Also, a p-aminosalicylic acid-bovine serum albumin antibiotic conjugate had superior efficacy compared to the free drug when tested in murine macrophages as well as a guinea pig TB infection model.

Previously, we demonstrated that chemical modification of glucans with carboxymethyl groups leads to their selective uptake by tissue macrophage scavenger receptors of the A type (ScR-A). In this study, we prepared a conjugate of the antibiotic moxifloxacin with carboxymethylglucan (CMG), investigated the targeted delivery of this conjugate to infected macrophages, and evaluated its antituberculosis activity. We will provide evidence that the moxifloxacin-CMG conjugate has enhanced uptake into macrophages and increased antimycobacterial activity relative to the free drug.

FORECASTING PANDEMIC INFLUENZA: PERIODIC RE-APPEARANCE OF HIGHLY VIRULENT H1N1 VIRUSES AND EMERGENCE OF NEW RECOMBINANT VIRUSES CAPABLE OF CROSSING INTERSPECIES BARRIERS AS A PROLOGUE TO PANDEMICS; NEW APPROACHES FOR DESIGN OF "GEOGRAPHICAL" AND NEW GENERATION POLYVALENT VACCINES

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Recent years have been marked by growing anticipation and fear of a flu pandemic caused by one of highly pathogenic strains of avian influenza (HPAI) virus. The recent emergence in Mexico and the United Sates of swine-origin influenza A H1N1 virus, capable of effective human-to-human transmission with rapid worldwide spread, caught health officials and epidemiologists off guard. Detailed genetic analysis indicated that the new virus was derived from several viruses circulating in swine and that its genomic segments have been circulating undetected for an extended period of time before the virus' emergence in humans. This is additional evidence that mixing, reassortment and, possibly recombination, of influenza genetic elements can lead to emergence of viruses with pandemic potential in humans. Several past pandemics, in particular those in 1957, 1968 and 1977, were caused by influenza viruses that originated in Southeast Asia. Recent isolation of avian-like H1N1 virus from pigs in China appears to have originated from European swine H1N1 viruses. New H1N2 reassortant viruses carrying gene segments from classical swine, human and avian lineages supports long standing hypothesis that swine can serve as "mixing vessels" for generations of pandemic influenza viruses. Current pandemic swine flu virus causes a mild infection, while an increase in its pathogenicity is anticipated and widely feared due to efficient human-to-human transmission. Reverse transmission from humans to pigs has been also documented. The highly contagious virus possesses two segments that originated from avian lineage. Its return into "the mixing vessel" may lead to further reassortment with HPAI and to better adaptation to mammalian hosts. These findings underscore the urgent need for systematic, worldwide surveillance of influenza in swine, especially along natural bird migration routes from Southeast Asia to Europe. Current project is designed to establish systematic sampling and characterization of influenza viruses from migrating waterfowl, from pigs in pig farms and human cases as follows:

1.To organize systematic collection of samples in Novosibirsk city area and oblast bordering the north-eastern part of Kazakhstan, Almaty city and region areas, and eastern Kazakhstan along routes of bird migration from China to Eurasia.
2. To detect and amplify influenza virus isolates in chick embryos, primary cell lines
3. To characterize and serotype virus isolates using hemagglutination inhibition, ELISA and neutralization assays with type-specific control reference sera.
4. To characterize virus isolates by genomic sequencing of segments amplified by RT-PCR and identify genetic variants with predicted increase in mammalian virulence and transmissibility.
5. To characterize virulence and transmission of selected influenza virus isolates with predicted increased virulence and transmissibility in the ferret model of influenza developed at the Southern Research Institute (Birmingham, AL, USA) under contract with NIAID.
6. To develop a predictive algorithm for the timely design of influenza vaccines effective against emerging viruses with epidemic and pandemic potential.

TB TEST-KIT FOR RAPID DRUG SUSCEPTIBILITY TESTING OF *MYCOBACTERIUM TUBERCULOSIS*

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Drug-resistant tuberculosis is an increasing public health concern in many parts of the world, including Russia. Traditional drug susceptibility testing is either time-consuming (21-28 days for the absolute concentration method) or expensive (automatic BACTEC systems). In contrast, the TB test-kit developed at SRCAMB (in the frame of BTEP/DHHS Partner projects #1846 and 2748) allows for reducing the time of testing in 2.5 times, has a low price and can be used in all the bacteriological labs. Performance of testing with the test-kit does not require special equipment or highly skilled personnel.

The TB test-kit is a kit of ready-to-use nutrient media with drugs or without them and reagents for reading results. The test-kit is able to determine the susceptibility of *M.tuberculosis* to isoniazid, rifampicin, streptomycin, ethambutol, and to perform primary identification of *M.tuberculosis*. Drug susceptibility testing with the TB test-kit is based on the ability of *M.tuberculosis* to reduce nitrates to nitrites. The nitrites are detected with Griess reagent, which produces a color change. Sputum or suspension of *M.tuberculosis* strains inoculates into each a vial of the test-kit with syringe. Then vials of inoculated media incubate at (35±2) ^{0}C. In 8-13 days of incubation resistant isolates of *M.tuberculosis* produce color changes in the vial with an appropriate drug. Susceptible isolates does not produce a color change.

In the frame of BTEP/DHHS Partner projects #1846 the pilot-industrial technology for producing the test-kit has been developed; the properties of the TB test-kit have been studied; the terms and conditions of the test-kit storage have been determined. In the context of BTEP/DHHS Partner projects 2748 the SRCAMB TB test-kit has been tested in three clinical laboratories in Russia in comparison with the absolute concentration method and the automated BACTEC MGIT 960 using about one thousand *M.tuberculosis* isolates. Good agreement between the results of TB test kit and two techniques was found.

In 2008, the RF Ministry of Health approved the TB-test kit for production, sale and distribution at Russian Federation. The TB test-kit is currently being manufactured in SCRAMB and sold by small lots. The basic consumers of TB test-kit are mycobacteriological laboratories.

THE METHOD OF ANTIVIRAL VACCINES PREPARATION AND CONSERVATION

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Description: The proposed technology concerns the fields of virology, medicine, biotechnology and veterinary medicine and should be applied to the antiviral vaccines' preparation and conservation especially for the anti-rabies and anti-influenza vaccines. With the scope of the inactivated vaccines' preparedness terms prolongation and preservation of the viral antigens from the autolysis (proteolytic splitting) during the storage period, it is proposed to introduce the stabilizer in the form of the proteolysis inhibitor – E-aminocaproic acid (E-ACA). It was experimentally shown that the animals immunized with the inactivated anti-rabies vaccine based on the rabies virus strain Vnukovo-32 and containing E-ACA, were by 21 % more protected from the lethal rabies infection caused by the rabies virus strain CVS (Fig.1). It could be seen directly that the E-ACA addition leads to the creation of higher level of rabies virus-neutralizing antibodies. The E-ACA introduction to the content of the inactivated anti-influenza vaccine causes its better storage and further higher level of immunogeneity (Fig.2). To support this hypothesis, we performed laboratory animals' immunization with the vaccines E-ACA containing and without E-ACA addition. Both types of the vaccines were stored for 6 months' period at the temperature of 4^{o}C. It was shown that the animals, immunized by the E-ACA containing vaccine, were more protected than those immunized with the regular vaccine. The stabilized vaccine causes the prevention of the infectious viral titers increase by 2,0 lg EID_{50} as compared to the regular vaccine without E-ACA addition.

These experimental results have shown that following animals' immunization with the E-ACA containing vaccine the antibodies titers were 1.86 times higher than those in animals vaccinated with the regular vaccine (Fig.2). The antibodies' level in serum taken from the animals vaccinated with the modified vaccine after 3 and 12 months of its storage was high enough. It was shown that under the conditions of the mice double vaccination with the E-ACA containing vaccine after the 3 months period of its storage, the virus-neutralizing antibodies' level in the vaccinated animals' blood serum were increased due to the vaccination by 2.2 times. Even the year-long period of the E-ACA containing vaccine storage, causes the 1.3 times increase of the anti-influenza antibodies level as compared to the corresponding value caused by the regular vaccine without the stabilizer.

Innovative aspect and main advantages: We have shown for the first time that E-ACA could be used successfully as the inactivated live vaccines' stabilizer. Its addition allows to:

- Diminish the vaccine allergeneity;
- Prolong the vaccine storage period;
- Prevent the viral antigens' destruction;
- Increase the vaccines' immunogeneity;
- Protect the viral antigens from autolysis.

Areas of Application: The proposed technology could be attributed to the field of medicine, biotechnology and veterinary medicine, namely to the methods of live inactivated antiviral

vaccines production. The vaccines, prepared with the use of the proposed technology could be used for the specific anti-rabies and anti-influenza prophylactics of humans and animals.

Stage of Development: The proposed vaccine preparation technology is laboratory tested and experiments were performed on the animal models. It is patented in Ukraine (Patent of Ukraine # 9574 "Method of Vaccine preparation" published 17/10/2005/, priority of 27/12/2004

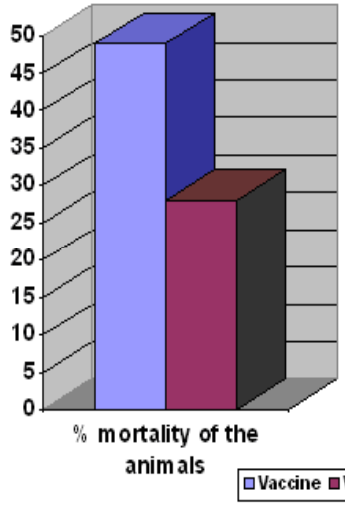

Figure 1: Influence of E-ACA on the protection efficacy of anti-rabies vaccine.

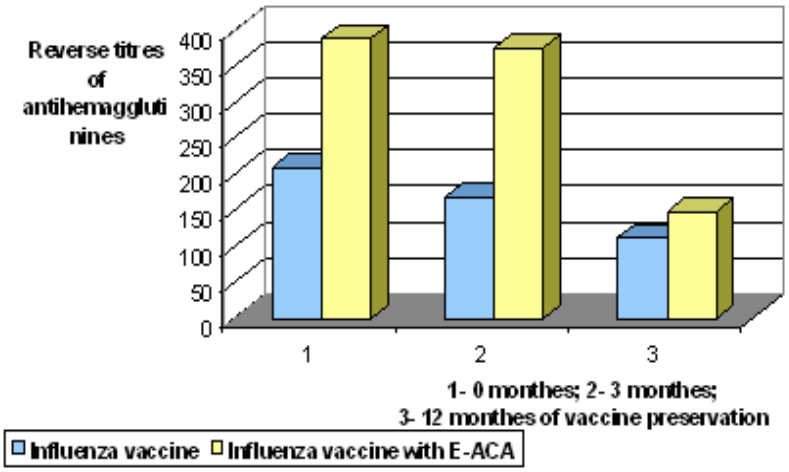

Fig.2. Influence of E-ACA on the titers of anti-influenza antibodies after immunization by vaccines with different preservation periods

DEVELOPMENT OF A RAPID TEST FOR CLINICAL LABORATORY DIAGNOSTICS OF *CHLAMYDIA TRACHOMATIS*

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The overall goal of our study is to develop a reliable and rapid immunodiagnostic test for the laboratory diagnosis of Chlamydia infection of urogenital tract (CIUT). For this purpose, we use two ELISA modifications: dot-ELISA on a nitrocellulose membrane (NCM) and immuno-chromatography (ICG). The ELISA-based methods are chosen because: i) they are significantly less expensive than the methods of molecular diagnostics; ii) the results are rapid (the duration is in the range of 30-90 min); iii) the methodology is simple and does not require the use of special costly equipment or trained personnel and can be performed in any poorly equipped laboratory; iv) the tests can be used for either a large-scale examination of human samples (dot-ELISA) or by patients themselves at home (ICG). To obtain Chlamydia-specific antibodies suitable for these tests, we applied the original technology developed by us previously, which involved a manipulation of the host immune response of inbred BALB/c mice to a single specific component of a Chlamydia antigen (C-antigen). The common platform of two immunodiagnostic methods will allow us to significantly improve the efficacy and diagnostic value of the immunoglobulin-based kits, which both employ murine mono-specific antibodies (MsAbs) to a chromosomally-encoded surface-located immunoreactive C-antigen. The main advantage of these MsAbs is their desirable specificity which is not restricted to a single epitope as characteristic to monoclonal antibodies. On the other hand, their specific antigen recognition is much narrower than that of Chlamydia C-antigen polyclonal antibodies. The use of the latter antibodies in diagnostics of Chlamydia often results in non-specific reactions. Thus, the use of MsAbs to C-antigen will result in a reduction in the rate of false-negative and false-positive results in diagnostic kits, which designed to simultaneously detect *C. trachomatis* strains without dependency on the presence of the Chlamydia cryptic plasmid as well as overcome a possible genetic polymorphism of Chlamydia antigens in clinical specimens of patients with CIUT.

To obtain the Chlamydia specific antibodies with the highest titer values to the C-antigen, we immunized separate groups of inbred BALB/c mice by using six different protocols and also rabbits (as a control) according to four different protocols. After the last immunization, the cells of myeloma line Sp 2/0-Ag.8 were injected intraperitoneally to the BALB/c mice primed with the “Pristane” in order to induce immuno-ascetic fluids, containing Chlamydia-specific antibodies. The immune rabbits were bled routinely using the aural veins.

In indirect ELISA with the C-antigen as a sensitin, specific antibody titers were: in the rabbits 1:5,120 (groups 1, 4), 1:1,280 (group 2), and 1:20,480 (group 3); in the murine ascetic fluids 1:400–1:25,600 depending on the immunisation schedule used with the maximal level in the group 4 (the short schedule).

To test the diagnostic value of the Chlamydia antibodies generated in our immunization experiments, we are in the process of creating a collection of clinical samples from patients with confirmed CIUT as well as from healthy donors. These specimens are analysed in dot-ELISA for evaluation of specificity of experimental series of murine and rabbit antibodies. MsAbs obtained by the protocols 1-4 are most active in dot-ELISA with the sera from patients with CIUT, while those obtained by the protocols 3 and 4 are better in recognition of the urethral scrapes. Rabbit antibodies are significantly less active than MsAbs with respect to both types of specimens. In parallel, these clinical samples are tested with two commercial ELISA test kits and in direct fluorescent monoclonal kit (DFMK). In general, MsAbs obtained by us demonstrate increased sensitivity and specificity over the commercial kits tested.

We specifically investigated the quality of the dot-ELISA results obtained with the use of different types of NCM purchased from a several vendors, and found that the membrane produced by BioRad (Hercules, CA) were optimal for detection of *C. trachomatis* in this modification of ELISA. To further develop both direct dot-ELISA and one-step ICG, immuno-conjugates of purified Chlamydia immunoglobulins are under preparation. The immunochemical purity of the batches of ascetic fluid and rabbit antiserum are confirmed by SDS-PAGE which typically showing a presence of two major bands with molecular weight of 25±2 and 50±2 kea corresponding to the light and heavy chains of immunoglobulin class G and occurrence of a several additional minor bands.

The immunoglobulin-based diagnostic kits developed by us will be used for a large-scale testing of clinical specimens from Chlamydia patients suspected for Chlamydia infection. Their diagnostic value will be compared with that of commercial immunoglobulin (ELISA, DFMK) and PCR kits. Currently, PCR-based kits target a cryptic multicopy plasmid which resides in *C. trachomatis* of different serovars. It was reported recently that fully virulent strains of *C. trachomatis* that lack the entire plasmid or its part are rapidly emerging in most parts of the Europe. Nothing is known about the distribution of such strains in Russian Federation; and current PCR systems, used to diagnose Chlamydia infection, will likely leave these strains undetected. Since our C-antigen based immunokits should be able to recognize these emerging strains of *C. trachomatis*, we hope to improve the PCR diagnosis of Chlamydia to account for this new threat for the human health.

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INVESTIGATION OF A VISCERAL LEISHMANIASIS FOCUS IN TBILISI, GEORGIA

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Epidemiological and entomological studies were carried out in an active visceral leishmaniasis (VL) focus in Tbilisi, the capital of Georgia. Serological tests were performed on children, ages 1-14 year, and stray and pet dogs living in the VL focus. Sand flies were collected in the same area using CDC light traps and sticky paper traps. Sand flies were identified using a collection of taxonomic keys and live female flies were dissected and examined for parasite infection. The direct agglutination test (DAT) was done on filter paper blood samples collected from 4,266 children. The results indicated baseline seropositivity equal to 7.3% (titer ≥1:6400) and a follow-up study (one year after the baseline survey) showed disease conversion in 6.0% of investigated children. Serum samples from dogs were screened for anti-*Leishmania* antibodies using the rK39 dipstick test. A total of 111 of 630 domestic dogs (17.6%) and 110 of 718 stray dogs (15.3%) were found to be seropositive. Presence of *Leishmania* amastigotes was confirmed by microscopy in 49 bone marrow aspirates from positive domestic dogs. Of five species of sand flies identified during the survey, the most abundant was *Phlebotomus kandelakii* (64%), followed by *Phlebotomus sergenti* (19%), *Phlebotomus balcanicus* (10%), *Phlebotomus halepensis* (6%), and *Phlebotomus wenyoni* (1%). *Leishmania* promastigotes were revealed microscopically in two species: *P. kandelakii* and *P. balcanicus* (infection rate ≈1%).

PCR analysis performed on bone marrow samples from clinically diagnosed children, serologically positive dogs and infected sand flies revealed infections of *Leishmania infantum.* The obtained results demonstrate a currently active focus of *L. infantum* in humans and reservoirs (dogs) and incriminate *P. kandelakii* as the most likely vector. These results also indicate the feasibility and need for determining the extent of active VL transmission and presence in all of Tbilisi in order to assess the overall public health risk. Further investigations are justified as follows:

1. Recent clinical evidence indicates the spread of human leishmaniasis cases to other parts of Tbilisi. Therefore to understand leishmaniasis transmission and determine the prevalence in human and canine populations it is important to extend this work to all areas of the city.

2. Detection of *P. kandelakii* and *P. balcanicus* naturally infected with *L. infantum* is critical evidence that more than one vector is responsible for transmission of this parasite. Expansion of vector surveillance in all sectors of the city through additional vector seasons is crucial to obtain adequate numbers of sand flies to verify our findings on vector species and diversity, VL transmission dynamics and to comprehensively define the vector(s) of VL in this area.

3. Several investigators have shown that an essential issue in the search for VL vaccines, and specifically new molecular targets for vector-based salivary vaccines, is investigation of Leishmania vectors. Thus, our plan is to investigate the saliva of sand fly species collected in Georgia to identify new molecular targets for an anti-vector vaccine against VL. Through this work useful vaccines for other regions where these sand fly species are proven vectors will also be identified.

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ISTC DRUG DISCOVERY LANDSCAPE

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The International Science and Technology Center (ISTC) is an intergovernmental organization created to serve the goal of non-proliferation. It was established in 1992 by the European Union, Japan, the Russian Federation and the USA on the basis of a multinational agreement to coordinate efforts of various governments, international and private sector organizations to provide new opportunities to the former weapons scientists from Russia and Commonwealth of Independent States (CIS).. The core ISTC activities are associated with science projects of scientific cooperation on a global scale. Since 1994 the ISTC has provided financial support, exceeding $826M to 2671 projects. Through ISTC partner program, ISTC Partners (private companies, government agencies and non-governmental organizations) directly fund, or co-fund R&D projects undertaken by CIS scientists and institutions. Partners working with ISTC affiliated scientists and institutes receive many advantages benefits. Partners gain the value of established ISTC infrastructure developed over 10 years, such as in-country project management (overcoming language barriers), customs clearance, Intellectual Property Rights (IPR) support and cost free search for their R&D needs. Partners have funded more than 580 projects worth $221 M in a wide range of technology areas. Some Partner projects concentrate on early stage R&D whereas others focus on obtaining a product ready for market. In the area of Drug Discovery more than a hundred of the projects have been funded for about $40 M.

Since 1998 ISTC is working jointly with governmental and business US partners (BioIndustry Initiative, Department of Health and Human Services, United States Department of Agriculture, Nuclear Threat Initiative, Defense Advanced Research Project Agency, Defense Threat Reduction Agency) through the Partner's and Parties programs on creation of a world's standard for GXP (GLP, GMP, GCP) in CIS. In order to reach this goal, efforts have been made to increase the capacity of animal-testing and production facilities in the institutes and ensure their compliance with international GLP and GMP standards by upgrading facilities, improving documentation-flow, auditing, monitoring and training of personnel, and formation of new inter-organizational networks. In 2007 ISTC Funding Parties approved priority for Targeted Initiative "Drug Design and Development" which strengthens and consolidates the odd elements of drug discovery R&D and infrastructure in CIS with a purpose to integrate it into the international market which includes following directions:

- Development of new platforms and assays for bio-screening;
- Production of highly-demanded biotarget molecules, cell lines and recombinant proteins;
- Development of GLP-compliant bio-analytical pre-clinical services and infrastructure;
- Development of GMP-compliant production of drug candidates for pre-clinical trials;
- Trainings/assistance in IPR handling, sales force, quality management;
- Information/communication support;
- Improvment of global community in the areas of excellence and drug discovery outsourcing opportunities in CIS

The ISTC has established strategic partnership with several international innovational foundations and organizations engaged in technology transfer, with other international institutions, government organizations and agencies, business schools, legal companies, foundations and investors for further reinforcement of economic sustainability of biotech organizations of the CIS.

HEPATITIS B AND C DIAGNOSTICS: DEVELOPMENT OF REFERENCE PREPARATIONS FOR EVALUATING THE QUALITY OF TEST KITS AND THE PROFICIENCY LEVEL OF THE CLINICAL DIAGNOSTIC LABORATORY STAFF

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The practical task is to develop such standards that would reliably evaluate the efficiency of screening and confirmative test kits. Composition of the standard will correspond to a set of infectious markers, the concentration of each marker being near the lower limit of its medical content. Some problems of viral hepatitis diagnostics can be solved only with the adequate reference preparations, in particular, Control and Master serum panels containing HBsAg and anti-HCV antibodies. In current practice, control tests are used, which include EIA screening followed by confirmation with the aid of more specific methods, such as Western blot, neutralizing test, or NAT. Quality control of hepatitis B and C diagnostics includes two main problems: the level of professional skills and the quality of serological assay performance and data processing.

For hepatitis B, the marker is HBsAg and two main subtypes AY & AD. Accordingly, Working Standard consists of two vials with HBsAg and main subtypes AY & AD to control the sensitivity (by titration data), and one vial with donor serum to control the specificity. The quality of test kits intended for HBsAg detection can be assessed with the Master panel developed under the ISTC/BTEP grant 1803p; this Master panel is composed of human blood sera having HBsAg content from 2.0 to 0.05 IU/ml. Positive part of the Master panel includes 200 samples divided into 3 blocks: the subtypes block, the sensitivity block, and the block of undetermined samples. For control of the test kit specificity, negative part of the panel comprising 170 samples was supplemented with donor sera and sera from the risk group members.

Analysis of the Master panel application with different test kits showed that a standard national panel for practical State control should be composed of a set of MP samples by the following procedure: 1.After the screening, to perform statistical processing of the OD data obtained for K^+ and K^- samples. 2. To calculate Cut off and EIA grey zone from the values of (OD_{av}^+) and (OD_{av}^-) and their root mean square errors (s^+ and s^-). 3.To limit the size of a commercial standard panel by 24-32 samples in order to control the results by at least triple measurements on the same plate. 4.To test the standard panels and calculate the errors in determination of analytical sensitivity (Se) and specificity (Sp).

Standard serum panels containing anti-HCV antibodies have been used for evaluating the quality of anti-HCV test kits at the Tarasevich SISC since 1995. At present, the developed anti-HCV serum panel lot 014HC is being certified. The panel comprises 24 serum samples: sera with a low content of antibodies (which is typical of clinical practice), sera that have antibodies only to Core- or NS-proteins, and sera containing HCV markers of 1a, 1b, 2a and 3a genotypes. To control the test kit specificity, 8 negative sera are included in the panel.

A necessary condition for increasing the efficiency of hepatitis B and C diagnostics is State control over the quality of serological assay performance, which depends not only on the test kit quality, but also on the proficiency of laboratory personnel. Russian Federal System of External Quality Evaluation at the RF Health Ministry includes the Program of certifying the clinical diagnostic laboratories regarding the quality control of hepatitis B and C assays. Within the Program, encoded serum samples of control panels are delivered to the laboratories being certified.

Analysis of results obtained from nearly 2 400 serological laboratories in the period of 1997–2002 allows prompt identification of main problems in hepatitis B and C practical diagnostics. One of the potential problems with validation of standard preparations is that HCV infected serum is a multi-component system in which anti-Core, anti-NS3, anti-NS4 and anti-NS5 antibodies are detected simultaneously. Due to the absence of a general International anti-HCV standard, there is no experimental method for comparison of the data obtained with different EIA kits and in different laboratories. The Working group of World Health Organization (WHO) on biological standardization made an attempt to artificially create a single-component standard for HCV antibodies, but this approach proved to be inefficient (WHO, Geneva, October 2003 [8]). In Project 3526p we have developed service programs for statistical evaluation and processing of EIA data and suggested that multi-component serum samples could be validated using the statistical analysis of these data.

The next step of Project 3526p is the development of a Master Panel (MP) composed of samples obtained from different regions of Russia and distributed into three blocks. The first block will comprise samples certified for HCV genotypes, 1a, 1b, 2 and 3. The second block represents the samples titrated in the range from native form to Cut off, and the third block includes samples with analytical indetermination. This third block consists of serum samples in which anti-HCV is detected not by all test kits. The following conditions criteria were used for inclusion in this block: positive for HCV RNA, and elevated levels of aminotransferases. Negative block of MP consists of 100 serum samples which were anti-HCV and HCV RNA negative. Five Russian patents for the methods of standard serum panels production and certification were obtained. The studies performed under ISTC/BTEP Projects 1803 and 3526 formed a basis for the development of standard preparations to be used for State control over the quality of viral hepatitis B and C diagnostics. We propose the follow-up research Project, which consists in arrangement of the production line complying with GMP requirements, certification of the products, and promotion of the pilot-production lots of reference preparations for quality control of hepatitis B and C diagnostics to the Russian and CIS market. The production bay and laboratory will be organized at JSC MBU with the involvement of specialists from SRC VB Vector. 29 new working places are to be established under the Project, among them 19 working places for former weapon scientists (ex-WMDs).

Consumers of reference preparations are government and private laboratories of hospitals, blood banks, AIDS Centers, manufacturers of ELISA test kits and government regulatory authorities (the Tarasevich SISC, Federal program of external quality control). During nearest years the market value will increase due to enlarging the menu of reference preparations and increase amount of laboratories. At a present time JSC MBU has established distribution network in Russia and CIS countries. Standard panels of sera are supplied for Government order in the framework National Priority Project “Health”. Manufacturing of reference preparations is highly profitable business (profitability is about 80%). Our forecast of sales value is up to 2.000,000 USD per year.

Hierarchic QSAR technology for Effective Virtual Screening and Design of Perspective Drug Agents

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Description: Hierarchical QSAR technology (HT) is destined for optimization of new effective drug agents creation process. HT allows us to solve the QSAR task not *ab ovo*, but with the use of information received from a previous stage by mean of the system of improved solutions. The unique and principle feature of the HT consists in multiple-aspects hierarchical strategy that related to (Fig 1-2):

- models of molecular structure description (1D → 2D → 3D → 4D);
- models of atoms description in molecular simplexes (descriptor physical -chemical → field);
- structural descriptors (local → integral);
- scales of activity estimation (binary → nominal → ordinal → continual);
- mathematical methods of analysis the structure-activity relationship (pattern recognition→ rank correlation → multivariate regression → PLS → SVM → "Random Forest");
- final aims of QSAR research (prediction interpretation → structure optimization → molecular design).

The set of the different QSAR models that are supplementing each other is the result of application of HT. These models all together, in complex, solve the problems of virtual screening, evaluation of structural factors influence on activity, modification of known molecular structures and design of new high-performance potential drug agents.

HT corresponds fully OECD Principles for the Validation, for Regulatory Purposes, of QSAR models. The Developed HT has been realized arranged as a complex of Software tools.

Innovative aspect and main advantages: Simplex representation of molecular structure, that is providesing universality, diversity and flexibility of description of compounds related to different structural types.; Iin addition, there is a unique possibility of QSAR analysis for mixtures of compounds.

HT use depends on concrete aims of research and allows to construct generation the of optimal strategy of QSAR models, avoiding the superfluous complications that do not result in an increase in accuracy.

HT does not have the restrictions of such well-known and widely used approaches as CoMFA (Comparative Molecular Field Analysis), CoMSIA, and HASL, usage of these programs lasts is limited in to the structurally homogeneous set of molecules and only one conformer.

HT also does not have the HQSAR shortcomings that are related to the ambiguity of descriptors system formation. Furthermore, on every stage of HT usage we can determine the molecular structure features that are important for the studied activity, and exclude the rest. It

shows unambiguously the limits of expedient QSAR models complication and allows not to waste superfluous resources on needless calculations.

The efficiency of the HT was shown on an example of compounds that possess antiviral activity. In an initial (training) set there were only 12% of active compounds, but after application of the HT, there were 75% of new designed compounds turned out an prospective antiviral compounds agents (see for example Patent of Ukraine №61292A, priority of 20.12.2002).

Figure 1:

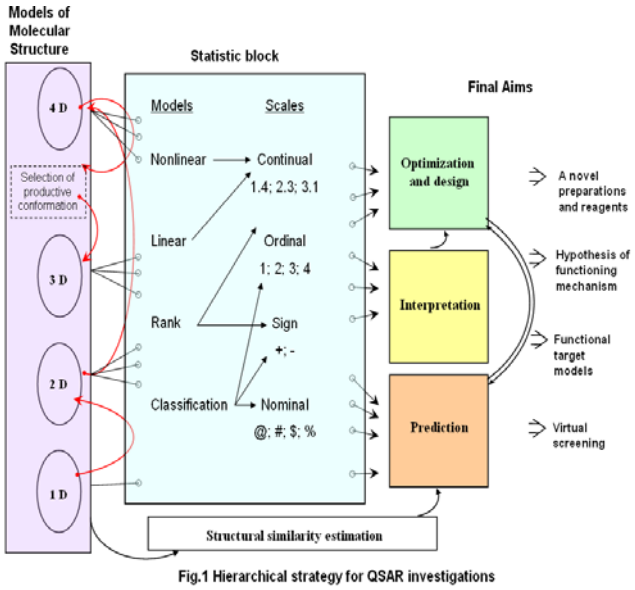

Fig.1 Hierarchical strategy for QSAR investigations

Figure 2:

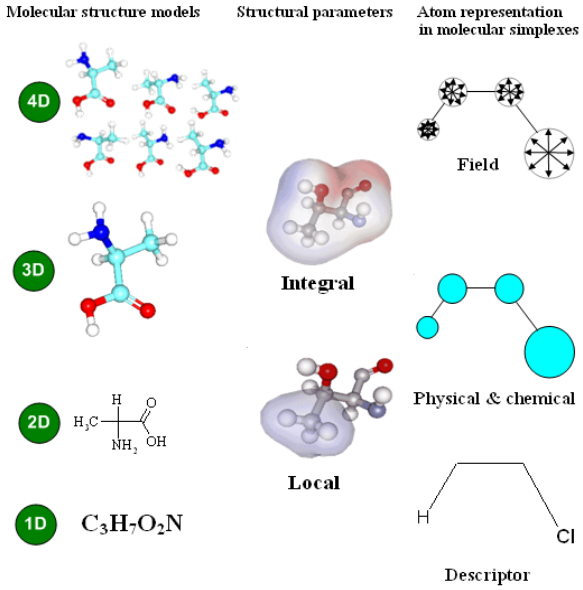

Fig.2. Hierchical system of molecular structure representation

CANDIDATE VACCINE CAPABLE OF ELICITING A BROADLY REACTING ANTIBODY RESPONSE TO STRUCTURALLY CONSERVED AREAS ON THE HIV-1 V3 REGION

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Background: The main challenge to the development of anti-HIV vaccines arises from the extremely high genetic variability of the virus.

Methods: Our approach is aimed at eliciting a response to the specific areas on the V3 region that are "structurally conserved" across HIV strains. We have developed a chimeric peptide library (CPL) mimicking the antigenic variety of existing and potential variants of the HIV-1 subtype B third variable region. The library was created using an original antigenic similarity matrix to select amino acids thatmimic the heterogeneity of variable positions.

Results: The CPL-based candidate vaccine induced a marked humoral immune response to a broad range of both existing and potential variants of the V3 region in both mice and rabbits. To study the breadth of reactivity, we designed a representative peptide panel (RPP), containing 35 peptides, which represent the least probable variants of antigenic diversity of the existing and potential variants of the HIV-1 V3 region, for all subtypes. The cross reactivity of anti-CPL sera with RPP peptides demonstrate that most rabbit sera react strongly with almost all peptides of the RPP. Thus, immune response to the candidate vaccine was characterized by broad specificity, covering almost all subtypes and main virus variants. This indicates that immune responses to the conserved higher order motifs of V3 region were elicited in several models.

Conclusions: The data suggest that CPL-based candidate vaccine elicits pronounced potentially protective and therapeutic humoral immune response to a broad range of HIV-1 variants. It may be a valid approach for vaccine development, providing desirable cross-reactivity, especially for therapeutic HIV/AIDS vaccine development, because of its ability to prevent viral mutation in the V3 region.

Acknowledgements:

We thank the US DHHS Biotechnology Engagement Program for research grant (BTEP 34 ISTC 2450) and Bio-Industry Initiative (BII) program of the U.S. DOS for patenting support.

IPR ISSUES IN ISTC PARTNER PROJECTS

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Partner projects represent a special type of ISTC projects where outside financing of the project is fully provided by a partner while the ISTC Parties do not make any direct contribution to the financing. The ISTC partner is an organization officially approved by one of the ISTC Parties and has received confirmation of the ISTC Governing Board for participation in a partner program on a permanent basis. In the process of distribution of rights on intellectual property (IP) created in a project (both partner and regular, i.e. financed by the Parties), the ISTC is guided by two basic principles: 1) IP distribution should be fair and unbiased and should be based on the proportional contribution (both financial and scientific and technical) of the parties who signed the project agreement. 2) ISTC Parties welcome the patenting and wide dissemination of results of scientific research. While regular projects the agreed distribution of IP is fixed in a standard project agreement, with partner projects such distribution is determined by the Recipient and the partner on an individual basis. In each case, consideration is given to the specific conditions of the project, based on the basic principles specified above. Such an approach is also formulated in the ISTC Statute (Article XIII E). In addition, Partner orders and fully finances the project and therefore has a right to own the created IP. Furthermore, it should be taken into consideration that the translation of project results to a commercial stage, at which earnings are received, requires significant additional efforts, both financial and organizational. As a rule, such efforts are made by the Partner and therefore they must be considered in the process of negotiating the distribution rights and remunerations. At the same time, it should be taken into account that the Recipient-institute, even though it does not contribute any funds directly to the project, does make a financial contribution in the form of provision of equipment and devices, for which no money is charged, as well as in the form of overhead expenses and payments to social funds.

Generally, the Partner reserves more substantial rights. However, it is presumed that the Recipient receives adequate compensation for dividing up the rights for IP. It is also presumed that the inventors and authors receive royalties and other remuneration proportional to their contribution to the creation of IP. In case of equal contribution of a partner and a Recipient, the appropriate distribution of rights for IP can be achieved by retention of Recipients' rights on its territory, while the partner receives the rights on its territory, and both parties hold negotiation on such rights in third countries. In some cases, joint ownership of rights in one or several countries is preferable, or it could also be beneficial if one of the parties owned all the rights on a worldwide scale, having granted licenses to another party in one or several countries. Regardless of the final distribution, these issues should be discussed at a reasonably early stage, so that it could be specified in the project agreement in order to avoid complications that could occur in the process of IP rights distribution once such property is created. In partner projects the Center and each ISTC Party retain the rights to non-exclusive irrevocable royalty-free license (with the right to sublicense) in all countries for non-commercial use in articles, scientific and technical magazines, reports and books prepared as a result of each project. If neither the partner nor the Recipient provide for protection of the invention, neither the ISTC nor the ISTC parties will receive any rights for IP created under the project but they can disseminate information on it.

EXPERIENCE OF A BIOLOGICAL TESTING LABORATORY IN THE PHARMA/BIOTECH-INDUSTRIES

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The safety evaluation of pharma/biotech-industry products is based on comprehensive preclinical studies of toxicity. The preclinical safety package includes: acute and repeated dose toxicity, genetic toxicity, reproductive and developmental toxicity, carcinogenesis, safety pharmacology (involving three key organ systems: central nervous system, cardiovascular and respiratory systems), pharmacokinetics and toxicokinetics. These preclinical studies must be carried out in compliance with Good Laboratory Practice (GLP) principles. Principles of GLP apply to all non-clinical health and environmental safety studies, which are required to register or license many kinds of products: pharmaceuticals, pesticides, food and feed additives, cosmetics and veterinary drugs, and for the regulation of industrial chemicals. GLP is a quality system concerned with the organizational process and the conditions under which non-clinical health and environmental safety studies are planned, performed, monitored, recorded, archived and reported.

There are international GLP-standard (GLP OECD) and national GLP-standards (for example, GLP FDA and GLP EPA in the US). Currently in Russia there is no GLP-standard and there are no certified GLP-compliant laboratories. Nevertheless, in the Biological Testing Laboratory (BTL) of the Branch of the Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Science there are the, people, premises and operational units that are necessary for conducting non-clinical health and environmental safety studies.

The head of BTL has the authority and formal responsibility for the organization and functioning of the test facility according to the principles of GLP. In the BTL there is a Quality Assurance Program. This defined system includes personnel, and is independent of study conduct. It is designed to assure test facility management compliance with the principles of GLP. Every study in the BTL has a Study Director who has the individual responsible for the overall conduct of research, as well as a Study Protocol. This document defines the objectives and experimental design of each study and includes any amendments. The BTL has a Master Schedule, which is a compilation of information to assist in the assessment of workload and for tracking ongoing studies. In the BTL there are documented Standard Operating Procedures which describe how to perform tests or activities normally not specified in detail in study protocols or test guidelines.

The BTL is the first laboratory in Russia to receive accreditation by the Association for Assessment and Accreditation of Laboratory Animal Care. (AAALAC). Here, we demonstrate the feasibility of analyzing of pharma/biotech-industry products and industrial chemicals in compliance with GLP principles in Russia.

PROBIOTICS WITH BIOENTEROSEPTIC PROPERTIES FOR OPTIMIZATION OF HOST IMMUNE RESPONSE

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Western counties and CIS countries are facing a progressive increase in immune-mediated, gut-related health problems, such as allergies and autoimmune and inflammatory diseases, bacterial overgrowth syndrome, and these rapid increases in disease occur on a background of wide use of an oral probiotics and food supplements with probiotics. Modulation of the intestinal microbiota may be achieved by consuming living bacteria or by consuming a combination of probiotics and prebiotics. Probiotics based on Lactobacillus do not justify hopes assigned to them frequently. This can be due to their fast elimination and antagonism with host strains of Lactobacillus. Intake of probiotics and prebiotics results in stimulation of beneficial bacteria and their increased distribution in the host gut. At the same time they help in selective decontamination of pathogenic microorganisms in the gastrointestinal tract. In contrast, the use of antibiotics for these purposes contributes to simultaneous destruction of both pathogenic and beneficial microbiota in the host gut. Use of antibiotics for these purposes contributes to simultaneous destruction of pathogenic and host gut microbiota.

Probiotics preparations based on bacteria of the genus *Bacillus* from family Bacillaceae are effective for selective decontamination of the gut tract from pathogens, as representatives of the group "self-eliminating antagonist". The genus *Bacillus* is usually associated with soil, but its representatives are widespread everywhere and also in water, a dust, air. Human exposure to these bacteria occurred through millennia, i.e. throughout human evolution. Throughout human existence as a biological species, bacteria of the genus Bacillus were present in large quantities in human digestive tract and respiratory airways. The spectrum of antagonistic, enzymatic and immunomodulatic properties of aerobic spores bacteria considerably surpass all other probiotics. A spore-forming bacterium is capable of preventing digestive disorders, even to a greater degree, than traditional probiotics on the basis of Lacto- and Bifodobacterium. Their antagonism to the broad range of pathogenic microorganisms and their independent elimination from the digestive tract, does make the designing of treatment and prophylactic preparations from Bacillus especially prospective.

At FSRI SRC VB "Vector" we have designed a recombinant strain of *B. subtilis* (rB.subtilis) with inclusion of the gene of human a2-interferon. This strain is antagonist to pathogenic microflora and being a producer of interferon, possesses antivirus properties. On the basis this strain, we have developed effective food supplements and prebiotics preparations that eliminate pathogen microflora and stimulate gut host microflora. The probiotics strain of rB.subtilis renders immunomodulating action: it restores the immune status broken by pathology, increases production of host interferon and other cytokines, strengthens functional activity of macrophage and NK, and raises phagocyte activity of leukocytes in the blood. These preparations at use in a natural way in an organism (oral),eliminate of pathogenic microflora and stimulate gut host microflora, positively regulate the normal functioning of the gastrointestinal tract, the physiological functions and biochemical reactions of the host. The oral introduction of rB.Subtilis was shown to have prophylactic and therapeutic effects during an acute experimental infection of VVEE in pigs, and influenza A/PR8/34/H1N1 in the mouse model. The

antineoplastic and antimetastatic effect of the rB.Subtilis was shown on the mouse model of the lung carcinoma (LLC) and B-16 melanoma. The limited clinical conditions have shown, that during a dysentery, elimination of bacterial antigenes (Sh.Flexneri, Sh.Sonne) occurs within 5 days of use the probiotic based on rB. subtilis. Within 6 days of using of the rB.subtilis for therapy of certain liver diseases (hepatitis B, C and mix), the virus antigens are eliminated from the blood; essential decreases in levels of bilirubin and growth of the specific antibodies are observed. Considering the above facts to be established, we offer potential investors an opportunity for mutually favorable cooperation and partnership for the promotion of these preparations in a wide range of practices (biotechnology, veterinary, agriculture, medicine etc).

PROSPECTS OF NATURAL FOCI INFECTIONS SERODIAGNOSIS BY USE OF PHOSPHORESCENT ANALYSIS-BASED MICROPLATE MICROARRAY TECHNOLOGY

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In this study we focused on the development of phosphorescent immunochips on the basis of microplate microarray technology of Phosphorescent Analysis[1] (abbreviated PHOSPHAN) and used them for differential serodiagnosis of seasonal natural foci diseases caused by Tick-Borne Encephalitis (TBE) virus and Lyme Borreliosis (LB) agents. Immunochips were prepared as microspots 0.5 - 0.7mm in diameter "printed" on a microwell bottom of 96-well microplates and covered with immunoreagents (monoclonal antibodies, recombinant or peptide antigens) capable of specific binding of IgM and IgG antibodies against the agents of viral (TBE and WNF) and/or bacterial (LB, syphilis, and leptospirosis) infections.

Immunoassay was performed in a microwell similarly to currently used ELISAs. Water-soluble Pt coproporphyrin capable of long-lasting phosphorescence at a room temperature was used for streptavidin labeling. The phosphorescence was registered in time-resolved mode by scanning the bottom of preliminarily dried microwell with specialized scanner. Investigations were performed on a panel of well characterized sera collected in highly endemic Cisural region of Russia within the period of seasonal activity of vectors from patients with serologically confirmed clinical diagnosis. Diagnostic sensitivity and specificity of immunochips was compared with that of commercial ELISAs using ROC-curves analysis. The ability of immunochips to differentiate antibodies against different agents was evaluated by comparing P/N values with homologous and heterologous antigens. In most cases, the coincidence of PHOSPHAN and ELISA results was excellent and exceeded 95%. The main advantage of immunochip as compared with ELISAs is connected with its ability to detect antibodies against up to 16 (currently) or 36 (in the future) different antigens simultaneously that is important both for seroepidemiologic investigations and serodiagnosis of infections.

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THE DEVELOPMENT OF ANTI-FLU VACCINE EFFECTOR AS A DERIVATIVE OF THE LIPID-PROTEIN RAFT NANO-COMPLEX FROM INFLUENZA VIRIONS ENVELOPE

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This study concerns a new approach to influenza virus vaccines development. In addition to previously used killed and live influenza virus vaccines, we propose to develop a sustained immune response that can be achieved by subcutaneous or intranasal administration of liposome-like preparations containing cholesterol (immune-stimulating complexes, ISCOM's) and virus-like particles.

Our approach assumes that the viral envelope is an assembly of raft-like platforms composed of M1/HA/NA complexes and lipids, the platform stability results from highly-organized protein-protein interactions. Influenza virus envelope fragmentation by a cold mixture of two non-ionic detergents has been previously used to isolate specifically viral surface glycoproteins and matrix M1 protein as a raft containing lipid-protein complexes from purified viral preparations. The influenza A virus lipid bilayer was found to be cholesterol- and sphingomyelin-rich and thus partitioned preferentially into the lipid rafts.Two established methods of membrane protein isolation were combined: (i) the method of viral envelope glycoproteins selective extraction from intact virions by octyl-glucopyranoside (OG) at room temperature as a general approach to obtain split viral preparations, and (ii) the conventional method of isolation of detergent-resistant membranes (rafts) by cold solubilization of cell membranes with Triton X-100[2]. These raft domains obtained by either one of these methods differ from the total membrane fraction in their composition and their properties.

The association of the viral envelope glycoproteins and M1 into a raft lipid-protein complex was verified via detergent insolubility experiments. The M1:HA stoichiometry of the proposed supramolecular complex was estimated by amino acid analysis. Dynamic light scattering and electron microscopy data revealed that these lipid-protein rafts are compsed of single particles of ~28 nm. These particles also form unilamellar vesicles with HA spikes on their surfaces similar to the original influenza virus virions. Together, our data suggest that the cold co-extraction technique visualizes the raft-like nature of the viral envelope and demonstrates the interaction of matrix M1 protein with the envelope. Pilot immunization tests with preparations obtained by these methods using subcutaneous administration in mice showed ~50% immune activity compared to the whole-virus vaccine.

STUDY OF HEAT SHOCK PROTEIN FUNCTIONS IN LYMPHOID CELL POPULATIONS

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We study heat shock proteins (HSPs) in lymphoid cell populations, particularly the nature of extracellular pools of HSPs. We also study the immunomodulatory effects of HSPs on the immune system. The most attention is focused on the major HSP family, HSP70. We are also developing more sensitive assay system for soluble HSPs. These studies involve extensive use of flow cytometry and confocal microscopy to study these multifunctional proteins.

As a result, a novel approach to cytometric analysis of progressive stages of programmed death of lymphoid cells has been developed. A relationship exists between HSP70 expression and translocation to cell surface and key events of lymphocyte apoptosis, including transmembrane mitochondrial potential, caspase-3 activity, intracellular ROS concentration, Bcl2 and p53 expression. Our data suggest that HSPs play a protective role for lymphocytes, but are also important for the mediation of the death process. During apoptosis, cytoplasmic HSP70 was found rapidly translocate to the cell surface, and is released into the surrounding extracellular space. Inhibitors of protein transport and cell stress factors increased levels of HSP70 exocytosis to the cell surface and the extracellular media, indicating a Golgi-independent, non-classical transport pathway. Both T and B lymphocyte subsets showed this surface translocation phenomenon, and it is believed to occur at a slower rate during normal lymphocyte homeostasis. Surface HSPs constitute a recognition system for removal of apoptotic cells, and exogenous HSPs may play an intercellular signaling role during cell stress and apoptosis.

We also demonstrated that extracellular HSP70 possessed immunomodulatory properties. In particular, different lymphoid cell types can internalize exogenous HSP70, resulting in resistance to apoptosis. Extracellular pools of HSP70 also inhibited ROS production by phagocytic cells. The obtained results displayed that effect of extracellular HSP70 on immune system might be due not only to interaction of the protein with antigen-presenting cells but also to a direct HSP70 impact on signal transduction in a variety of immune cell types.

Analyzing HSP concentrations in cells and supernatants remains a difficult problem for cell biologists. The existing immunodetection systems (i.e. ELISAs) are expensive and have sensitivity problems. To address this, six B cell hybridomas producing anti-HSP70 monoclonal antibodies to different epitopes of the molecule have been developed, and are being used in a soluble HSP70 microsphere-based multiplex detection system. Sensitivities of 2 ng/ml or less have been obtained using this assay system.

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DEVELOPMENT OF AN OLIGONUCLEOTIDE MICROCHIP FOR TYPING VARIOUS SUBTYPES OF INFLUENZA VIRUS A

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Type A influenza virus circulating in the human population, as well as domestic and wild animals, presents a serious danger as a potential cause of a pandemic resulting from emergence of new influenza virus strains with unusual antigenic properties. An example is emergence of the avian influenza virus H5N1, which is highly pathogenic for fowl and humans. The reference and clinical laboratories must continuously monitor the antigenic shift and drift in the circulating virus strains to determine the vaccine currently necessary for vaccination.

The efficiency of hybridization microarray chips for typing influenza virus essentially depends on the methods for selecting the probes specific for the analyzed DNA. We have developed an original method searching for the typing probes able to determine the subtypes of influenza virus haemagglutinins and neuraminidases.

Eventually, we designed a microarray chip that is able to type influenza virus A according to haemagglutinin and neuraminidase genes. We have tested the selectivity of the designed probes typing the influenza virus neuraminidase and haemagglutinin genes using the available amplicons of human influenza viruses and, in part, avian influenza viruses. Two parameters were used to ascribe a sample to particular subtype according to the microarray data: (1) mean (normalized) fluorescence of spot (the sum of spot fluorescence intensities of a subtype divided by the number of spots) and (2) the fraction of fluorescing spots (the number of spots of a subtype displaying the fluorescence exceeding the mean fluorescence value for all spots of the microarray). The developed microarray chip correctly determines the analyzed subtypes of type A influenza virus.

The proposed method can be used for screening of the influenza virus reassortants obtained for influenza vaccine production; rapid diagnostics of natural reassortants, including the viruses belonging to different species; and monitoring of the antigenic drift within the same serotype.

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MICROBIOLOGICAL SYNTHESIS OF NEW MEDICINE PREPARATION WITH TONIC, ANTI-OXIDANT AND ANTI-TUMOR ACTIVITY

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The presented work is focused on microbiological transformations of natural phyto-ecdysteroid-ecdysteron (20-hydroxyecdyson) isolated from *Silene tatarica* (*Caryophellaceae* family) to obtain new compounds with a tonic, anti-oxidant and anti-tumor activity.

The limiting stage in the process of working on this project is the preparative production of the starting compound-ecdysteron- for subsequent microbiological and chemical research, as the content of target steroid in the plant material does not exceed 0.1% in terms of the mass of air-dry plant material. Also the chromatographic separation of the CO_2 extract of *Silene tatarica* the fractions with associated natural compounds that could possess novel, clinically relevant biological activities could be missed.

In connection with the above mentioned facts, we have initiated the search for alternative sources of ecdysteron from the plants of the following families: *Caryophyllaceae, Chenopodiaceae, Malvaceae, Asteraceae, Ranunculaceae*, *Liliaceae*, which grow on the territory of the Republic of Kazakhstan.

We are currently isolating mono-, sesqui-, diterpenoids and phenolic compounds and further fractionating these extracts for biological activity screening of individual compounds, as well as the original extracted substances at the National Cancer Institute, USA.

In collaboration with scientists at the National Cancer Institute, USA we are defining unique biological activities of submitted substances and determining the structures of lead compounds by NMR spectroscopy, elemental analysis, and X-ray analysis.

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BUILDING A FLOW CYTOMETRY CORE RESOURCE IN RUSSIA: A TEN-YEAR COLLABORATION BETWEEN THE INSTITUTE OF BIOORGANIC CHEMISTRY AND THE NATIONAL CANCER INSTITUTE

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Flow cytometry is a central technology in the life sciences, allowing the rapid analysis of single cells in large numbers. Flow cytometry has permitted detailed study of the complex cells of the immune system, and has been used to characterize tumor cells and study a variety of human diseases (such as HIV/AIDS). Fluorescence-activated cell sorting, an extension of flow cytometry that allows physical separation of complex cell mixtures, has allowed detailed study of purified rare immune cell populations, as well as genomic and proteomic analysis on purified cells.

The Laboratory of Cell Interactions at the Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry (SOIBC) and the Flow Cytometry Core Laboratory at the National Cancer Institute (NCI) have collaborated extensively for over ten years on a variety of problems in immune cell biology. To facilitate these studies, a core facility of fluorescence analysis instrumentation has been established at SOIBC. Beckman-Coulter XL and BD Biosciences FACScan benchtop flow cytometers were donated to SOIBC by the National Cancer Institute in 2002 and 2004 through the NCI Foreign Donation Program. A Varian scanning spectrofluorimeter was purchased for the facility through International Science and Technology Center regular project funds (ISTC 2185), and a Nikon laser scanning confocal microscope through an ISTC Partner Project with the Biotechnology Engagement Program (ISTC 2627 / BTEP 73). This collection is a valuable resource for ISTC-funded investigators, and is available to all biomedical investigators in the Moscow region, as well as former weapons scientists working on ISTC funded projects. It also provides an important education and training resource for graduate and post-graduate students.

This collaborative effort is in the process of its most ambitious donation yet, a BD Biosciences FACSVantage DiVa cell sorter, scheduled to arrive at SOIBC before the end of 2009. This instrument will allow physical separation of immune cell populations, filling a critical need for Moscow based investigators. As with previous donations, SOIBC and NCI investigators will work closely to provide maintenance and quality control for the donated equipment, as well as comprehensive education and training. The intent is to develop a sustainable research resource, facilitating collaborative research between the USA and Russia.

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EPIDEMIOLOGIC AND LABORATORY METHODS OF RUBELLA SURVEILLANCE IN RUSSIA

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Rubella is a disease that is particularly dangerous for pregnant women, because of its potential to cause multiple abnormalities during fetal development. It is known that the rubella virus has teratogenic properties. When virusemiya at pregnant women a activator can infect the placenta. Multiplying in it, and entering the fetus, followed by dissemination of organs, leading to a breach of organogenesis, causing the pathology in children known as congenital rubella syndrome (CRS). CRS is characterized by a multiplicity of lesions. Thus, in 60-70% of cases a combination of two or more developmental defects are found. Intrauterine infection often leads to spontaneous abortion and stillbirth (40%), when infected in the first 8 weeks of pregnancy. Rubella causes a large number of postpartum complications in pregnant women; such as: subacute rubella panencephalitis, type I diabetes, thyroiditis, etc.

In connection with the extreme relevance of this problem, rubella is a disease of great social significance. The main preventive measure is rubella vaccination (rubella live vaccine strain of the Ra 27/3). In Russia, vaccine prophylaxis was introduced into the national immunization calendar in December 1998, and actual immunization of children and women of childbearing age was carried out from 2002-2003. In only the last few years, attention has been given to the issues of epidemiological and laboratory surveillance for rubella. The final diagnosis of “Rubella”, or “Rubella in pregnancy,” or “CRS” for a child may be placed only on the basis of epidemiological and laboratory particularly rubella virus and immunoenzyme analysis (ELISA) studies. Practical healthcare of Russia needs highly specific and sensitive methods to diagnose «Rubella», and to differentiate it from similar clinical conditions. To this end, in practice, confirmatory serologic methods for epidemiological and virological studies are used. Thus, the verification of a clinical case requires the deployment of the virus, identification by PCR, and serologic confirmation of ELISA Ig M, low-and highly prominent anti-rubella IgG antibodies.

In recent years, the Russian Federation in the verification of rubella and CRS, except the usual for the country genotype 2C (which circulated widely in its territory until 2003) there have been recorded cases of rubella, caused by strains of the first genotype (1 E and 1 G). As evidenced by the build-up of the role of multiple imported cases from neighboring countries, and confirms the improvement of the epidemiological situation in Russia.

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BIOCHIPS AS A VALUABLE TOOL TO IDENTIFY DRUG-RESISTANT *MYCOBACTERIUM TUBERCULOSIS*.

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The gel-based biochips developed in Engelhardt Institute of Molecular Biology, RAS consists of hemispherical (150 μm in diameter) hydrogel elements which are arranged on a hydrophobic surface at the density of 10 units per square mm. The gel elements could bear immobilized nucleic acids, proteins, other biomolecules and even live bacterial cells. Biochips is a powerful tool for simultaneous analysis of number of genomic loci to identify mutations and single nucleotide polymorphisms. The hybridization biochip bears immobilized oligonucleotides or DNA fragments which interact specifically with the complementary sequences of the analyzed DNA target. To perform biochip-based analysis, the amplified and fluorescently labeled DNA/RNA specimen is injected into a biochip microchamber where the hybridization or PCR takes place. Specific fluorescence hybridization signals are captured and processed by a specialized biochip reader that is able to analyze any types of biochips produced by EIMB.

One of the most important clinical application of biochips is genomic analysis of the TB causative agent. It is extremely important to obtain results of antimycobacterial drug resistance in short time due to growing number of MDR (multidrug resistant) and XDR (extremely drug resistant) tuberculosis cases.

The TB-biochip is used to identify mutations responsible for rifampin (Rif) and isoniazid (Inh) resistance of Mycobacterium tuberculosis strains. These mutations are located in the rpoB, katG, inhA genes and in the intergenic regulatory region of the ahpC-oxyR genes. The technique allows detection more than 95% of Rif-resistant and about 80% of Inh-resistant MTB strains in clinical samples within twenty four hours.

Another microarray (TB-biochip-2) was developed to reveal fluoroquinolone (FQ)-resistance in M. tuberculosis isolates and clinical samples. The method allows one to identify 8 mutant variants of DNA in FQ-resistant strains (about 85% of all resistant forms). The sensitivity and specificity of the developed approach upon testing clinical samples were 93% and 100%, correspondingly.

Both TB diagnostic systems were approved by Russian Ministry of Health and certified to be applied in clinical practice. TB-Biochip and TB-Biochip-2 kits are already used in seventeen antituberculosis institutions in Russia, as well as in USA and Kyrgyzstan laboratories.

NOTES