INFECTIOUS DISEASE RESEARCH AT RIGA STRADIŅŠ UNIVERSITY
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INTRODUCTION

The World Health Organisation (WHO) stresses that infectious diseases are not only spreading faster, but also appear to be emerging more quickly than ever before. There are now nearly 40 diseases that were unknown a generation ago. WHO Statistics 2008 stress that about 25% of all annual deaths in the world are due to infectious diseases. The European Commission has always emphasized the importance of research on these issues.

The EC’s Seventh Framework Programme has specific topics in the health programme dedicated to the field of infectious diseases, designed to improve existing approaches for preventing, diagnosing, treating and controlling infectious diseases, as well as developing new ones based on new understanding of micro and macro mechanisms. The European Research Area (ERA) will facilitate the accomplishment of these objectives.

Infectious disease research has traditionally been an important element of health research in Latvia. In the last 20 years, however, the development of research has been uneven, partly because of difficulties in keeping pace with technological developments and due to reduced scientific knowledge exchange. The BALTINFECT project aims to unlock the research potential in the area of infectious, immunological and rare diseases at Riga Stradiņš University.

This action plan is consistent with priorities for fundamental and applied research in Latvia adopted by the Cabinet of Ministers of the Republic of Latvia. The strategic objectives of the BALTINFECT project are directly linked to this plan. Therefore, broad support for the objectives of BALTINFECT is present in Latvia, strengthening positive public opinion about European Framework programmes and, especially, the upcoming Horizon 2020. The project addresses knowledge transfer with other EU research teams and information exchange on current research with teams around the world. In this way, RSU is progressing towards a centre of excellence on infectious diseases research and reinforcing strong local cooperation in the Baltic area by restoring ties with immunologists in Poland and Lithuania and molecular biologists in Estonia. Knowledge exchange on research methods is also planned with researchers from other institutions in the ERA.
INSTITUTIONS,
RESEARCH FIELDS
HISTORY OF INFECTIOUS DESEASE RESEARCH AT RSU

The study of infectology in Latvia began in 1921 when students from the University of Latvia began training in a bacteriology course. Over the years, structural units developed in the research of infectious diseases. In 1950, when Riga Medical Institute was founded, infectious disease research became one of the main research fields.

Institutions currently involved in infectious disease research are A. Kirchenstein Institute of Microbiology and Virology, the Laboratory of Clinical Immunology and Immunogenetics, and the Department of Infectology and Dermatology.

A. Kirchenstein Institute of Microbiology and Virology (MVI) was founded in 1946 and initially focused on research in microbiology. Over time, RSU MVI developed virological investigations and gained international recognition with its research on infectious hepatitis, influenza and oncogenic viruses. The advanced research at the Institute resulted in some of the most valuable patents in Latvia – antiviral components Larifan and Rigvir. A special field of study at MVI is human herpes viruses types 6 and 7 and parvovirus B19.

The Laboratory of Clinical Immunology and Immunogenetics is the central institution for immunology research and study in Latvia. It was established in 1980. In 2009 the laboratory gained international certification in quality standards for research laboratories. The laboratory performs immunological and immunogenetic analysis of human and animal material, as well as studies on the MHC/HLA immunogenetic related to various diseases.

The Department of Infectology and Dermatology was founded in 1945. The Department integrates research in public health, infectious disease clinics, poverty-related infectious diseases, tropical diseases, and rare diseases of unclear aetiology. Recent research encompasses Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS), infectious and non-infectious diseases in dermatology and STS, and liver diseases.
Hematopoietic stem cell transplantation (HSCT) is an important method for the treatment of malignant hematological diseases such as Hodgkin’s lymphoma, non-Hodgkin’s lymphoma and multiple myeloma.

In the case of successful HSCT, viral infection remains one of the main causes of post-transplant morbidity and mortality. CMV is the known pathogen in post-transplant complications development with well-documented clinical studies of active infection. Although HHV-6 is considered to be an important opportunistic pathogen for patients who have undergone HSCT, the clinical significance of reactivation of this virus remains controversial. The clear association of HHV-7 with complications after HSCT has not been recognized. At present the question of potential interaction between beta-herpesviruses in a case of concurrent infection is not clear.

The aims of the study are to examine: 1) frequency of beta-herpesviruses (CMV, HHV-6 and HHV-7) reactivation during the early period after autologous peripheral blood stem cell transplantation (auto-PBSCT); 2) potential interactions between virus reactivation and the development of post-transplant complications; and 3) potential relationships among the viruses in regard to their reactivation during co-infection. The study is performed in cooperation with the Chemotherapy and Hematology Clinic at Riga East Clinical University Hospital.

Although all patients received prophylaxis against viral infections, the frequency of beta-herpesviruses reactivation are considerably higher after transplantation than before, and HHV-6 and HHV-7 are prevalent in patients with concurrent infection. These data suggest that antiviral prophylaxis is not sufficiently effective to prevent the reactivation of these viruses. At the same time, the recurrence of CMV is relatively low because CMV is more sensitive to valacyclovir. Results show that these viruses are effective modulators of the immune response, mainly by modulating the production of proinflammatory cytokines.

Chronic allograft nephropathy (CAN) is the leading cause of progressive renal failure and the most important cause of post-transplantation graft loss in renal transplant recipients. The viral infections, including beta-herpesviruses (cytomegalovirus [CMV], human herpesvirus -6 and -7 [HHV-6, HHV-7]) and polyoma virus [BKV]) infections, that widespread in the general population, are risk factor for CAN development.

The aims of our study are: 1) examine the beta-herpesviruses infection prevalence, 2) determine period activation of each the viruses during the post-transplantation period, 3) evaluate possible effects of the viruses’ activation on CAN development, and 4) evaluate the optimal duration of antiviral prophylaxis or preemptive therapy to prevent the viruses activation in renal transplant patients.

For the first time in Latvia, the investigation of BKV infection prevalence and activation and its relationship with CAN development in renal transplant recipients is carried out. The significance of active parvovirus B19 infection in the development of anemia in renal transplant recipients is studied. The logistic regression analysis of the data reveals a significant relationship between active B19 infection and severe rHuEPO therapy-resistant anemia development after RT.
Epidemiology, pathogenicity of human bocavirus (HBoV) species and possible association with lower respiratory tract illnesses and acute gastroenteritis in children – Z. Nora-Krūkle, S. Grāvelsiņa, S. Rasa, I. Ziemele, D. Gardovska, M. Murovska

Lower respiratory tract infections, frequently caused by viruses, are one of the leading causes of morbidity and mortality in children worldwide. In developing countries each year, up to 1.8 million children die due to acute respiratory tract diseases. Only around 40% of lower respiratory tract viral illnesses have an etiologically identifiable agent.

Human bocavirus (HBoV) is a new respiratory virus also associated with gastroenteritis in children. HBoV has been detected not only in respiratory and stool samples, but also in serum, tonsillar, saliva and urine samples as well as in river and sewage water.

No research to date has been completed on the four types of HBoV and its distribution in the Latvian population. This study will research molecular epidemiology, serology and genetical analysis for HBoV. To date there has been some analyses on HBoV presence in children under 4 years with acute respiratory tract infections or acute gastroenteritis hospitalized at the Children’s Clinical University Hospital. Blood samples, nasopharyngeal aspirates, and feces are collected and analyzed for the presence of HBoV. HBoV is present in hospitalized children with acute respiratory tract infections and gastroenteritis in Latvia. To clarify the pathogenic role of HBoV, further studies are required.
Virologic and morphologic evidences of human parvovirus B19 infection in rheumatoid arthritis and osteoarthritis –

The role of parvovirus B19 (B19) infection in etiopathogenesis of rheumatoid arthritis (RA) is controversial. B19 could be the cause of the initial immune process that promotes the development of RA in predisposed humans and it may influence the RA clinical course – disease activity and aggressivity. There are data that viral infections could be involved also in the pathogenesis of osteoarthritis (OA) which advanced stage often manifests with severe inflammation and lesions of joint tissues related to the B19 infection.

This study aims to determine the association between B19 infection activation and RA clinical outcome and to demonstrate the spectra of synovial and supporting tissue damage in OA patients in association with the presence of B19 infection markers.

In comparison of the RA patient groups with active and latent B19 infection, significant differences in DAS, CRP level, RF and anti-CCP levels are observed. In almost all RA patients with active infection, anti-B19 IgG class antibodies (especially anti-B19 NS1 antibodies) are found, which may indicate persistent B19 infection.

The data show the association between B19 active infection and exacerbation of RA, as well as advocate the involvement of B19 infection in etiopathogenesis of RA.

No difference in the B19 specific IgG antibody prevalence is found between OA patients and the control group. However antibodies to B19 NS1 are detected in 41.7% of OA patients vs. 3.7% in healthy persons. Findings of B19 specific sequences in OA patient synovial tissues DNA are not unequivocal, although B19 capsid protein expression was demonstrated in OA patient synovial, cartilaginous and osseous tissues. B19 infection may contribute to the inflammatory and structural damage in the advanced stage of OA. The results show that serological and molecular biological B19 infection markers should not be considered solely. These findings should be jointly implemented by immunohistochemistry data reflecting the local tissue damage and contribution of B19 to OA.
Beta-herpesviruses (HHV-6, HHV-7) and parvovirus B19 infections as possible risk factors for the development of autoimmune thyroid disease – Z. Nora-Krūkle, S. Rasa, S. Grāvelsiņa, A. Sultanova, M. Čistjakovs, S. Čapenko, E. Cunskis, V. Groma

Thyroid diseases are widespread. Data show HHV-6, HHV-7 and B19 persistence in thyroid gland tissue, as well as association of acute B19 infection with HT development in children. It is necessary to explore the influence of viral infection in etiopathogenesis of thyroid gland diseases.

Research is carried out investigating blood, plasma and tissue samples from patients with various etiology thyroid diseases, establishing the presence and activation of viruses. Also, immunohistochemical studies are carried out to prove their presence in thyrocytes. The main lymphocyte subpopulations are separated to show the load differences of herpesviruses in blood and thyroid gland tissue.

Results demonstrate undeniable HHV-6 and HHV-7 involvement in etiopathogenesis of thyroid gland diseases. It is interesting to note that, only HHV6-B type is found. B19 involvement in etiopathogenesis of thyroid gland disease is not confirmed.

Continuing research could be linked to interaction with herpesviruses (HHV-6 and HHV-7) and chemokine CXCL10 expression level. Increased CXCL10 level is associated with the development of autoimmune diseases. Herpesviruses can affect this chemokine expression by inducing INF-, which is one of the main factors to induce CXCL10.
Investigation of etiopathogenesis of myalgic encephalomyelitis/chronic fatigue syndrome, post-infection and unspecified origin encephalopathy and elaboration of diagnostic criterions –

Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) belongs to the so-called dysfunctional conditions with medically inexplicable symptoms, including dysfunctional cognitive disorder or encephalopathy (EP). ME/CFS has no effective standardized and reproducible diagnostic tests, or treatment or prevention strategies. Therefore ME/CFS differential diagnostics are intensively researched and specific biomarkers identified: viral activation, immunological changes, cytokine expression, molecular markers and marker genes.

The aim of our study is to determine the role/involvement of beta-herpesviruses (HHV-6A, HHV-6B, and HHV-7) and parvovirus B19 infection in etiopathogenesis of ME/CFS, post-infection and unspecified encephalopathy, and to identify objective biomarkers for the diagnosis of the disease.

108 ME/CFS patients and 90 healthy persons are enrolled in the study. Viral genomic sequences are detected by PCR, virus-specific antibodies and cytokine levels – by ELISA, HHV-6 variants – by restriction analysis. A definite relationship is observed between active beta-herpesvirus infection and subfebrility, lymphadenopathy and malaise after exertion, and between active B19 infection and multi joint pain. Neuropsychological disturbances are detected in all patients. The high rate of active HHV-6, HHV-7 and B19 infection/co-infection with the simultaneous increase in plasma pro-inflammatory cytokines’ level as well as the association between active viral infection and distinctive types of clinical symptoms shows necessity of simultaneous study of these viral infections for identification of possible subsets of ME/CFS.

PCR is used to detect of B19, HHV-6, HHV-7 DNA in autopsy specimens (dura and pia mater, brain tissue) of 34 individuals with and 34 without signs of encephalopathy and to define the HHV-6 variant. It is shown that meningeal and brain tissues are sites of B19, HHV-6 and HHV-7 persistency. Simultaneous study of these viral infections and their activity stages are required to identify the relationship with the development of encephalopathy.
In the EU, there were around 74,000 new cases of NHL and more than 31,000 deaths from NHL in 2008. Statistical data shows that NHL comprises about 75% of all lymphomas, and that the vast majority (about 85%) of NHL represents B-cell type of NHL. The causes of lymphoma are still unknown.

We analyzed expressions of CCR1 and CCR2 in B-cell lymphoma (BCL) cell lines, in EBV-positive Burkitt’s lymphoma (BL) and EBV-negative BL and BCL cell lines. We revealed that the mRNA expression of CCR1 and CCR2 is induced in EBV-positive BL cell lines compared with the isogenic EBV-negative cells. However, the cell surface expression of CCR1 and CCR2 is only detected in the small proportion (3-15%) of the cells in all of these cell lines. Moreover, aberrant CCR2 transcripts are found in 4 BL cell lines. We also have demonstrated that CCR1 and CCR2 are highly expressed on the CD19+CD10− cells in the peripheral blood of 4 patients with low-grade B-cell LPDs, in contrast to highly malignant B-cell lines. We propose a role for the 3p21.31 CCRs deficiency in a high-grade B-cell lymphoma pathogenesis and suggest that CCR1 and CCR2 may have prognostic relevance in lymphomas of activated B-cell type.

This study will provide insights into association of the cell-surface chemokine receptors CCR1 and CCR2 with driving the B-cell malignancy progression, thus providing a scientific basis for biomarker discovery and future preventative strategies.
Immunomodulators vs. antibacterials (Glycomune) –
V. Saulīte, S. Doniņa, S. Rasa, L. Peškova, J. Jermolājevs

Over the last 70 years, efforts to prevent or treat infectious diseases with antimicrobial drugs has been extraordinarily successful, but it has also resulted in the generation of multiple-drug-resistant (MDR) microorganisms. An alternative could be developing therapeutic and/or prophylactic strategies based on immunomodulation.

As immunomodulators do not act on microorganisms directly, they may fix the problem of emergence of resistance to antibacterials, broaden treatment options for immunocompromised patients and offer a broad spectrum of activity against viral, fungal and bacterial diseases. They could provide nonspecific emergency-treatment options against novel pathogens or in complex cases of oncological diseases.

Our study will assess the immunomodulatory effects of Glycomune Beta Glycan on cellular immunity in patients with malignant genital and lung tumors after surgery and/or radio- and/or chemotherapy and determine TK level changes in serum before and after use of the preparation.

After a four-month long therapy using Glycomune Beta Glycan, an increase in the number of natural immune cells are detected more frequently, which gives evidence of the most effective induction of these immune response components with beta-glucans. Beta-glucan activity could also be associated with the ability to induce CD4+ T-Ly increase. After treatment with Glycomune Beta Glycan, a reduction in thymidine kinase levels are found that could indicate a decrease in tumor cell division.

Our next project deals with development of a new product – an immunomodulator with antibacterial properties as an alternative to antibiotics for treatment and prophylaxis of mastitis in cows.
Aptamers as potential agents for detection/diagnoses of malignant B cells – I. Holodņuka

In cancer treatment, the biggest problem is targeting the specific cancer cell to deliver therapeutics that in the most cases are toxic. The cancer-cell-specific antibodies that are currently being used as drug-carriers cause major immune response. The application of the nucleotide modified RNA-aptamers, highly-specific, highly-affinitive and non-immunogenic, will allow to bypass these problems and to develop approaches for the specific targeting of B-cell lymphoma cells.

The aim of the project is to isolate the nucleotides-modified (nuclease protected) RNA-aptamers specific and high-affinitive to the cell surface molecules that differ in the malignant B-cell lymphoma cells and non-malignant B cells and to investigate their potential application as diagnostic/targeting agents in B-cell lymphoma (BCL).

The expected main goal of this study is implementation of the selected specific nmRNA-aptamers for the detection/diagnoses of malignant B cells in the blood of patients with B-cell lymphoproliferative diseases.
DynaFector is the device used in laboratory studies to provide a more effective transport of therapeutic substances into human cancer cells, particularly if therapeutic agents have been developed on the basis of DNA or siRNA. The principle of the device is based on a periodic magnetic field action on the superparamagnetic nanoparticles (SPIONs) coupled with DNA or siRNA, which are added to growing cells. The magnetic field attracts the nanoparticles together with the therapeutic substance, concentrating them on the surface of cancer cells promoting more effective intracellular uptake of these complexes. The advantage of the device is that the magnetic field periodically shifts under growing cancer cells. Due to time varied action of the magnetic field, the process of SPION sedimentation onto cell surface occurs in a stepwise manner with displacement in the x-y plane that significantly increases nanoparticle-cell surface receptor interactions and enhances the cellular uptake of therapeutic substance containing nanoparticles.

The new developed method of entry superparamagnetic nanoparticle is as follows: in comparison to the standard magnetofection method, DNA/siRNA complexes using this device allow both more efficient transfect monolayer and suspension culture cells and entering more SPIONs per one cell. This apparatus allows reaching the highest transfection efficiency of cancer cells and is currently one of the best means to evaluate the therapeutic effect of siRNA in vitro.
One of humanity’s greatest health problems – AIDS – appeared at the end of the 20th century. Knowledge on the influence of genetic factors on the progress of the disease, as well as on the limit of molecular genes of genetic polymorphism of HLA II class locus and on encoding process presentation antigen determinants in T cells is insufficient.

The aims of our study are to confirm the influence of HLA class II antigens on the progression of HIV infection and to assess a possible relationship between these antigens. To appraise association of genetic polymorphism of HLA II class loci DRB1*, DQA1*, DQB1* with the body protection ability during infection, we have used the RT-PCR-SSP test, which permits the routine determination of HLA class II distributions. We have analysed the medical documentation of 2500 patients and have included 1180 HIV infected patients.

A prevalence of genes DRB1; DQA1; DQB1 and DRB1-DQA-DQB1 combinations in five groups of HIV infected patients are determined. Comparative analysis is also performed in the control group. The histocompatibility complex enables marker functions that can be used in additional diagnostics in cases of HIV infection. The results indicate that upon identification of HIV genes, it is possible to understand the molecular mechanisms in cases of progression of AIDS; this can possibly serve in the determination of clinical results of infected patients.
Lyme disease is caused by infection with the tickborne bacterium *Borrelia burgdorferi*. The human pathogen, *B. burgdorferi*, causes a multisystem disease that can affect the skin, nervous system, heart, or joints. The disease level in Latvia is one of the highest in Europe.

The purpose of this study is to determine MHC -DR, -DQ haplotypes in patients with clinical, epidemiological and laboratory diagnosed Lyme borreliosis. The study includes 78 patients with erythema migrans and 100 control subjects. MHC genotyping is performed by PCR with sequence-specific primers (SSP).

The frequency of MHC haplotypes is significantly increased in the Lyme disease patients compared with the control group. These results suggest that the inflammatory events of the subacute arthritis can set the stage for development of chronic disease in individuals possessing risk haplotypes. In particular, the haplotypes -DRB1*15:01:01/DQA1*01:02:01/DQB1*03:02:01, and DRB1*01:01:01/DQA1*03:01:01/DQB1*03:02:01, contribute significantly to a genetic predisposition to *Borrelia burgdorferi* infection in the Latvian population, which may have implications in our understanding of pathogenesis of this disease.
The specific course of the disease might be explained by the diverse immunogenetic backgrounds of individual patients. The host ability to react to viral antigens has often been associated with the human leukocyte antigen (HLA), mainly HLA class II antigens. Many studies suggest that the cellular immune response, e.g. to HIV infection, particularly the T helper (Th) lymphocyte response, plays a crucial role.

Our research took into account the structure of this gene, and we found that the structural changes in the HLA gene may promote an inadequate and incorrect binding of a peptide and presentation to T-cells. We want to clarify the role of the amino acid replacement in the HLA-DRB1*0101 gene and whether it affects a more rapid progression of AIDS in HIV patients.

The aim of the research is to determine whether ongoing missense mutations in the exon 2 of DRB1*01:01 affect the operation of this protective allele in HIV patients. The study includes 200 HIV-infected patients. DNA is isolated from venous blood samples using the Qiagen QIAamp DNA kit reagents and the exon 2 nucleotide sequence of HLA is determined by automatic sequencing Big Dye Terminator mix.

We found missense mutations at codon 47 in 80% of cases; at codon 67 in 20% of cases; at codon 75 in 11% of cases; at codon 82 in 10% of cases; at codon 86 in 10% of cases. One of the HIV patients has a STOP-codon (codon 13). Besides, a balance between nucleotide transversion and transition is observed, suggesting mutations in the exon 2.

We found that amino acid replacement could increase the risk of faster development of HIV than AIDS. After the assessment of the risk and ratio of the protective alleles, it could be possible to determine which patient is predisposed to faster development of AIDS, and this in turn would determine which patient needs to begin treatment sooner, thus improving the quality of life and prolonging survival.
One of the ways to treat HIV/AIDS is antiretroviral therapy (ART), which consists of various methods and includes a number of drugs. However, in view of the HLA polymorphism, it is necessary to determine the best combination of ART with HLA class II haplotypes. The purpose of this current study is to evaluate various HLA class II haplotypes with ART effectiveness in HIV-infected patients.

This study is a follow-up on the association of ART with the HLA class II haplotypes of HIV/AIDS patients. Blood is collected from 254 HIV/AIDS infected patients and HLA class II haplotypes are defined. The main ART therapy parameters are observed and compared using immunological parameters: the amount of CD4+ lymphocytes and HIV viral load. For DNA extraction venous blood is used. HLA class II alleles DRB1*, DQA1*, DQB1* genotyping is performed using the RT-PCR method.

Our study has partly confirmed that specific HLA class II haplotype may possibly influence the ability of the organism to resist HIV infection. Gene alleles DRB1* 01:01, 04:01, 13:01; HLA-DQA1*01:03, 04:01, 05:01, HLA-DQB1* 03:01, 03:03, 04:01-2, 06:01, 06:02-8 are considered protective against HIV infection. These alleles provide more effective HIV epitope presentation to CD4+ T lymphocytes. As a result, the immune system resists the HIV infection more effectively.
Autoantibody prevalence in chronic Hepatitis C patients in Latvia – L. Vīksna, E. Hagina, U. Bekmane

Hepatitis C is a major health problem around the world. Various geographical regions are characterized by certain autoantibody prevalence in sera of Chronic hepatitis C (CHC) patients. Hepatitis C virus (HCV) type 2, 3, 4 infections have better prognosis than HCV type 1 related CHC. This study presents the results of autoantibodies detection in CHC patients in Latvia.

The aim of this research was to study autoantibody prevalence in CHC patients infected with various types of HCV.

Patients with epidemiological, clinical, serological, biochemical, morphological evidence of HCV infection and duration of hepatitis at least 6 months were examined for presence of autoantibodies before treatment. Five types of autoantibodies were tested in indirect immunofluorescence on rat tissues: ANA – antinuclear antibody, SMS – smooth muscle antibody, anti-LKM – liver-kidney microsomal antibody, anti-GPC – antibody to gastric parietal cells, AMA – antimitochondrial antibody (The Binding Site Ltd., England). The types of HCV were detected using Murex HCV Serotyping 1-6 Assay.

Our results indicate a high prevalence of SMA, average – of ANA and anti-GPC and low – of anti-LKM in CHC patients. Autoantibodies were more frequently found in sera of CHC patients infected by HCV type 1 versus those who were infected by HCV types 2-4. The patients with HCV type 1 could have higher risk of autoimmune component than the patients with HCV type 2, 3, 4.
The role of genetic factors in Juvenile Idiopathic Arthritis (JIA) –
V. Staņēviča, J. Eglīte

The routinely accepted practice to start with Sulphasalazine (SS) as the first line treatment in patients with HLA B27 positive JIA proves to be ineffective in a large number of children.

More attention is being paid to the HLA system because it is significant in polymorphous immunological reactions. Several studies have suggested that genetic susceptibility to JIA is linked to HLA classes I and II alleles.

We hypothesized that HLA classes I and II associations within JIA may be more consistent, if analysed amongst patients with a relatively homogeneous clinical outcome. We suggest that genetic variations within the chromosome 6p31-32 HLA –DR/DQ locus might be associated with JIA susceptibility in children. Furthermore, our study alludes to the potential role of chromosome 4q27 locus and INF–, PTPN22, TNF–, IL6, IL10 molecular genes polymorphisms in conferring this risk.

The aims of our study are to evaluate juvenile idiopathic arthritis clinical, genetic and immunological correlation, which would result in guidelines for JIA diagnosis, treatment and complications and would provide significant economic effect, and extend the study of gene polymorphism in the 4q27 locus and locus HLA(DQ/DR) 6p21.31- 6p21.32 and the expanded INF–, PTPN22, TNF–, IL6 and IL10 molecular genes polymorphisms in patients with JIA. We also use results from a previous study of DQB1 and DRB1 genotyping.

A total of 94 JIA patients under the age of 18 years are surveyed and analysed in Latvia. On the basis of genetic markers, we discover it is possible to detect the risk factors of JIA prior to clinical manifestations, predict the likely course of the disease and, most importantly, develop criteria for early JIA diagnosis and individual disease modifying antirheumatic drugs (DMARD) therapy to reduce the formation of disabilities. This would play an important role in improving the quality of life in patients with JIA – the most common rheumatic diseases in children. It would also reduce Health Department expenses on the diagnosis and treatment and reduce formation of complications.
The recognized number of important diseases transmitted by ticks has grown over the past 30 years. The incidence of Lyme borreliosis in Latvia is one of the highest in Europe. Clarifying the polymorphism of HLA immunogenetic molecular markers to identify regularities in the development and pathology is needed to develop a new approach to treatment of these diseases.

In order to identify genetic markers, we investigated the HLA DR alleles in two groups of patients: those with Lyme borreliosis and those with tick-borne encephalitis. Blood samples are collected and genomic DNA is extracted from proteinase-K-treated peripheral blood leukocytes.

We found that the frequency of HLA-DRB1*04 is significantly higher in patients with Lyme borreliosis and in patients with tick-borne encephalitis than in the control group (odds ratio, 2.58; p=0.03).

HLA antigens may act alone or in combination with other genes, conferring susceptibility to, or protection against, infectious diseases. The mechanisms of immune response to infection that are influenced by the HLA genes may be the key to future vaccines, using the peptides of organisms that mimic the HLA antigens.
Genetic background interaction between infectious agent and host – R. Ranka, I. Jansone, I. Pole, G. Šķenders, I. Ozere, A. Nodieva

The incidence of Tuberculosis (TB) in Latvia has decreased from 74/100 000 in 1998 to 36.2 in 2011 in Latvia. However the rate increased by 10% in 2012, including children with TB.

The goal of the study is to isolate and analyse *M. tuberculosis* (MT) genotypes in children and analyse possible infection sources and transmission routes among children.

Active TB transfer from child to child is rare and is limited to close family or social contacts. Molecular genotyping has confirmed the finding of contact investigations in 62% of cases. This points to the need to expand the search range. Significant proportion of unique genotypes (12%) indicates the existence of unknown sources of infection circulating in society, or possible infection transmission from outside.
The number of patients with combined human immunodeficiency virus (HIV) and tuberculosis (TB) has recently increased. There were 1055 TB/HIV cases registered in the EU in 2010; 71 in Latvia (9.5% out of all TB cases in 2010, in comparison with 0.5% in 2000). The treatment provided in Latvia for patients with TB/HIV is based on WHO recommendations, including DOTS strategy. However, TB treatment results for HIV positive cases are of concern due to increasing resistance to HIV/AIDS drugs, as well as resistance to TB drugs.

Treatment results for 7761 new smear and/or culture positive pulmonary TB cases are analyzed; 234 among them are HIV positive. In untreated HIV/AIDS patients, resistance to treatment is found in 5.3% of cases. In treated patients the resistance to different groups of drugs is detected in 41% of cases. Multidrug resistance (MDR) is diagnosed in 14% of TB cases. Molecular biological, immunofluorescence, bacteriological and bacterioscopic methods are used for detection of initiating agents. The TB treatment results for HIV positive and HIV negative cases reported from 2000-2007 are compared with the results reported in 2010. Data for 2000-2007 are grouped together due to small number of HIV positive cases within the period.

The treatment success for HIV positive cases is lower in both periods (60-61%) in comparison with HIV negative cases (78-76%). The level of MDR TB cases among HIV positive patients is higher (15%) than in HIV negative patients (8%) during 2000-2007, but similar in both groups (9-10,6%) in 2010. Despite improvements in HIV/AIDS treatment since 2000, the death rate among HIV positive cases in 2010 is higher (18%) than from 2000-2007 (11%). The resistance to HIV/AIDS drugs is a factor influencing TB treatment results.
The levels of apoptosis markers in different groups of HIV infected patients – I. Ekšteina, V. Sondore, L. Viksna, B. Rozentāle, A. Ivanovs, I. Zeltiņa, G. Stūre

HIV-1 infection is characterized by a progressive loss of CD4+ T cells. The role of apoptotic processes are identified recently, but limited information is available so far. The aim of this study is to compare levels of apoptosis markers – cytokeratin 18 neoepitope (CK18) and cytochrome C (CC) in different HIV infected groups.

There are 69 HIV infected patients enrolled in the study. They are divided into four groups according to CD4+ T cell count and presence of opportunistic infections (OI). Opportunistic infections include tuberculosis, cryptococcosis, CMV infections and PCP. The serum levels of cytokeratin 18 neoepitope and cytochrome C are determined. Comparisons between groups are made using paired T-tests.

The levels of CC are not significantly different between groups with CD4+ cell counts above or below 200 c/mcl. Levels of CC are not significantly influenced by the presence of opportunistic infections. We found significant difference of CK18 levels between the group without opportunistic infections and CD4+ cell count above 200 c/mcl (210,58 ±26,98 u/l) and the group without opportunistic infections and CD4+ cell count above 200 c/mcl (132,95±14,09 u/l), p=0,02, or between the group without opportunistic infections and CD4+ cell count below 200 c/mcl (132,95±14,09 u/l) and the group with opportunistic infections and CD4+ cell count below 200 c/mcl.

The results of this study demonstrated elevation of levels of apoptosis serum markers early in HIV infection, ancipitating further decrease of CD4 cell counts.
The aim of this study is to identify new non-invasive methods for assessment of liver function in acute and chronic liver disease and evaluate the clinical diagnostic and prognostic accuracy of these methods, to cover advantages and disadvantages of noninvasive alternatives to liver biopsy, and to share experience and impressions accumulated in the field of hepatology.

Diagnosis is based on modern immunochemical hepatitis marker assays and clinical, biochemical and morphological findings.

168 patients are enrolled in immunogenomic study and divided into four groups: those with HCV infection and treated with PEG-interferon + Ribavirin; HCV infection and treated with PEG-interferon + Ribavirin; HCV infection treated with Realdiron therapy; and patients with HCV infection treated with Realdiron (non-responders). In group E, 100 healthy donors are included as the control group.

The study showed very high serum level of CK-18 neoepitope in patients with acute hepatitis B – higher than in patients with alcoholic hepatitis. CK-18 neoepitope concentration in acute hepatitis C and chronic hepatitis C is significantly lower. About one-third of chronic hepatitis C patients have normal serum ALT activity, but elevated serum CK-18 neoepitope concentration. Normally, cytochrome C is not detectable in serum, but 47.45% of patients with chronic hepatitis C have increased levels of this apoptosis indicator. Serum concentration of cytochrome C are even higher in acute hepatitides of viral and toxic etiologies.

The timely identification of immunogenetic factors may prove to be useful in predicting disease evolution, in guiding the appropriate therapy for patients with poor prognosis and in encouraging the development of untherapeutic strategies.
Neisseria Gonorrhoea: antimicrobial resistance and risk factors in Latvia – I. Mikažāns, I. Upeniece, I. Saulīte

Decreased antimicrobial suspectibility and treatment failures with first line antimicrobial therapy for in vitro gonorrhoea in Latvia has become problematic because the incidence of gonorrhoea remains high and there are no new antibiotics available.

In our study, the Neisseria Gonorrhoeae isolates obtained from gonorrhoeae patients will be tested using GCII Agar with IsoVitaleX™ Enrichment 8-150 mm style plates and BBL, Sensi-Disc, for doxycycline, ceftriaxone, ciprofloxacin, gentamycin, azithromycin, cefixime, norfloxacin, ofloxacin, clarithromycin and cefotaxime. Additional patients’ data linked to the Neisseria Gonorrhoeae isolates suspectibility profiles will be collected. All antimicrobial susceptibility data and other related information about patients will be combined and patient variables associated with resistance will be established.

The results and outcomes of the study will play an important role in development of individualized diagnostic and treatment algorithm for gonorrhoea patients.
THE BALTINFECT PROJECT
The strategic objective of the project is the strengthening of multidisciplinary infectious diseases research in the Baltics, by unlocking the research potential at RSU.

RSU will improve the overall infrastructure by:

- unlocking and strengthening the research capacity in the area of infectious, immunological and rare diseases,
- establishing two new laboratories: a digital immunological visualisation laboratory and an infectious diseases modelling laboratory,
- upgrading the existing personnel capacity through increasing the level of competences in immunology visualisation and in-silico and mathematical modelling,
- upgrading research equipment,
- knowledge transfer between RSU and leading institutions in infectology in ERA,
- raising research personnel proficiency by employing experienced researchers to facilitate the integration into ERA,
- profiling infectious diseases as a smart specialisation of RSU,
- creating an intellectual property development plan,
- tying information activities to scientific workshops and conferences,
- improving technology transfer and research process management quality, and
- obtaining quality evaluations from the EC as an excellent facility in the region for participation in the Horizon 2020 programme.

To achieve this goal, effort will focus on the following:

**Management and coordination**

Organisation of smooth and timely implementation of all project activities, monitoring the project’s progress and reporting the outcomes to the EC; sound and reliable management of legal and financial issues, implementation of recommendations of the advisory board, efficient control of project risks.

A kick-off meeting opened the BALTINFECT project. The Project Steering Committee (PSC) will meet at the start of each project year and at the closing of the project (Round Table with evaluators). Permanent project staff is contracted based on the task schedule. Schedule of joint activities with partnering
organisations is updated with exact dates. Regular project management team meetings take place to ensure optimal and smooth implementation of BALTINFECT by identifying eventual risks and making adequate and optimal decisions. Reports on BALTINFECT progress will be delivered after 18, 36 and 42 months. A sub-contracted audit of financial statements will be carried out twice – after the completion of the project activities for M1-M18 and M19-M36. An advisory board delivered an initial opinion and will report annually on the achievement of the project’s objectives and attained results.

Exchange of know-how and experience
The main format of know-how and experience transfer (K&E) are direct two-way secondments between RSU and other EU institutions. The secondments are scheduled in relation to the research priorities of RSU in the following directions: research process management and knowledge transfer, imaging and new methods of visualisation in immunology, immunohistochemistry, new methods based on molecular biology, genomics, proteomics, bioinformatics, systems biology, immune system response to the viral infection, epidemiology of viral diseases, methods for rare diseases research. Two coaching courses are integrated into K&E activities. Courses were led by the strategic partner Steinbeis Research Center Technology Management Northeast and covered the issues of management of Intellectual property rights and research process management. Handbook of Research Process Management for RSU after the training course will be developed during months.

Recruitment of incomming experienced reserachers
Recruitment of experienced researchers for the period of 32 months. Preparation and accomplishment of competition for two experienced researcher positions: immunological visualisation and infectious diseases modelling were prepared.

Equipment upgrade
The underlying idea of the project is to advance the research level at RSU by investment into personal capacity and by equipment upgrade. With upgraded equipment the RSU will become more adapted for responding promptly to scientific challenges as well as to address important public health issues joining the EU “task force” on infectious diseases. The equipment and software for visualisation and modelling purposes in immunology and infectious diseases has been acquired.
Organisation of workshops and conferences
Knowledge transfer between RSU and other institutions will promote two part and multipart collaboration between institutions doing research in virology and communicable diseases in order to create strong research consortia in H2020 and to integrate the RSU into the ERA. There will be two scientific workshops and one international conference organized within the framework of the BALTINFECT project.

Dissemination and promotional activities
Creation and regular updating of a webpage that will report on progress of the project, on-going activities and opportunities identified in the project and collaboration proposals. It will contain links to regulatory acts related to the project and its public reports.

Publication and dissemination of brochures on RSU infectious diseases research and a special issue of the Proceedings of the Latvian Academy of Sciences (referred by SCOPUS) will be prepared.

The project will receive global recognition at a special RSU-BALTINFECT booth/poster presentation at major infectious diseases events.

A profile on RSU will be placed in the CORDIS partner database and distributed through the network of National Contact points. A special emphasis will be put on participation in H2020.

Special attention will be paid on RSU participation in informing industry, including SMEs, about immunology, technology transfer and industrial involvement.

Regular collaboration with public media to ensure flow of information. The BALTINFECT project will be reflected in 2 presentations of ca. 20-min length in broadcasting media: one in television and one in radio. Regular information updates will be distributed via press-services.

A special report will be produced about technological advancements in infectious diseases research, new diagnostics and therapies, future challenges and immunology frontiers. A popular science film will be produced on topical issues in infectious diseases research; challenges caused by viruses and bacteria and the role of outstanding researchers in infectious diseases research.

Targeted activities to integrate with ESFRI infrastructures in Translational research and Biobanking.

During the Latvian EU Council Presidency in 2015, RSU and the Latvian Ministry of Health will propose to devote one of the topics of the Presidency on infectious, rare and poverty related diseases.
**Intellectual property development**

An intellectual property development plan will be developed by consultants. RSU will offer support through the Technology Transfer Office (TTO). Special emphasis will be on IP management in biotechnology, biomedicine and clinical medicine.

A dedicated innovation scorecard will be developed for RSU in order to evaluate departments, institutes and individual researchers. Further pilot acceleration projects are planned providing direct advice in the pre-market phase.

**External evaluation**

Project evaluation will take place after the end of the implementation of the Action Plan. Evaluation will be carried out by independent international experts. RSU staff will prepare the necessary documentation. Discussion boards will be organised with representatives of Latvian ministries and agencies as needed. Results of the evaluation will be discussed at a RSU Round Table by leading staff, Steering Committee and Scientific Advisory board members, representatives from the Latvian Ministry of Health and Ministry of Education and Science and the Latvian Academy of Sciences.

The research potential increase of RSU in BALTINFECT Project will be realised in close cooperation with at least 10 research and technology transfer institutions from different EU countries. Three of them are outstanding partnering organizations with an especially important role in the project.
PROJECT PARTICIPANTS are:

<table>
<thead>
<tr>
<th>Country</th>
<th>Institution</th>
<th>Scientific fields</th>
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<tbody>
<tr>
<td>Latvia</td>
<td>August Kirchenstein Institute of Microbiology and Virology at Riga Stradiņš University</td>
<td>Virology, immunomodulators, oncoviruses and virotherapy, biomarkers, long-term biocompatibility.</td>
</tr>
<tr>
<td></td>
<td>Laboratory of Clinical Immunology and Immunogenetics</td>
<td>HLA biology, histocompatibility, population immunogenetics, immune status, biocompatibility, biomarkers.</td>
</tr>
<tr>
<td></td>
<td>Department of Infectology and Dermatology</td>
<td>Infectious diseases, poverty related diseases – TBC, AIDS, rare diseases, clinical trials.</td>
</tr>
<tr>
<td>Sweden</td>
<td>Uppsala University, Department of Medical Sciences, Section of Clinical Virology</td>
<td>Virology, detection methods, bioinformatics, modelling</td>
</tr>
<tr>
<td></td>
<td>Karolinska Institutet, Department of Microbiology, Tumour and Cell Biology</td>
<td>Immunobiology, biomarkers, visualisation centre</td>
</tr>
<tr>
<td>UK</td>
<td>London School of Hygiene and Tropical Medicine, University of London</td>
<td>Epidemiology, biobanking, visual immunology, rare infectious diseases</td>
</tr>
<tr>
<td></td>
<td>Buckinghamshire New University</td>
<td>Epidemiology, bioinformatics, mathematical modelling</td>
</tr>
<tr>
<td><strong>Country</strong></td>
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<tr>
<td><strong>Germany</strong></td>
<td>Steinbeis Research Center Technology Management Northeast</td>
<td>Technology transfer, IPR</td>
</tr>
<tr>
<td><strong>Lithuania</strong></td>
<td>Lithuanian State Institute of Innovative Medicine</td>
<td>Immunology</td>
</tr>
<tr>
<td><strong>Bulgaria</strong></td>
<td>Institute of Experimental Morphology, Pathology and Anthropology, Bulgarian Academy of Sciences</td>
<td>Infectious pathology</td>
</tr>
<tr>
<td><strong>Poland</strong></td>
<td>Medical University of Silesia</td>
<td>Infectious diseases modelling</td>
</tr>
<tr>
<td><strong>Belgium</strong></td>
<td>Katholieke University Leuven</td>
<td>HIV/AIDS, molecular biology of infections, phylogenetic analysis</td>
</tr>
<tr>
<td><strong>Belarus</strong></td>
<td>Centre of Epidemiology and Microbiology, Belarus Ministry of Health</td>
<td>Virology, epidemiological models</td>
</tr>
<tr>
<td><strong>Ireland</strong></td>
<td>University College Dublin, St Vincent’s University Hospital</td>
<td>Inflammation and cancer immunology</td>
</tr>
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</table>
PROJECT IMPLEMENTATION

The **advisory board** will provide advice on implementation and promotion of integration in the ERA.

**Maria Söderlund-Venermo**, Associate Professor, Department of Virology, Haartman Institute, University of Helsinki

**Thomas Schulz**, Professor, Director of the Institute of Virology, Hannover Medical School

**Dario Di Luca**, Professor of Microbiology and Clinical Microbiology, Faculty of Medicine, University of Ferrara

The **project steering committee** will carry out strategy and policy decisions.

**Iveta Ozolanta**, Professor, Vice-Rector of Science

**Jurijs Perevoščikovs**, Director of the Infectious Diseases Risk Analysis and Prevention, Centre for Disease Prevention and Control in Latvia


**Ojārs Spārītis**, Professor, President of the Academy of Sciences

**Frank Graage**, Steinbeis Team Northeast in Rostock

**Jonas I. O. Blomberg**, Professor, Section of Clinical Virology, Department of Medical Sciences, Uppsala University

**Luis Carlos Nacul**, Professor, London School of Hygiene and Tropical Medicine

The Baltinfect project management team will manage and coordinate project activities and monitor the progress of the project.

**Modra Murovska**, Project Baltinfect Coordinator, Associate Professor, Director, A. Kirchenstein Institute of Microbiology and Virology, modra@latnet.lv

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Linda Gabrusenoka, Head of Technology Transfer Office, Intellectual property development team leader
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CONCLUSION
NEW REALITIES, NEW OPPORTUNITIES

BALTINFECT will assign a new dimension to the collaboration in regional networks and will create conditions for the sustainable further development of broad mutual cooperation – even after the end of the project. During the project, networking activities will be opened to other participants in ERA as well.

An integral priority of the BALTINFECT project will be responding to the needs of the SMEs, involvement of the SMEs into project’s activities and wide networking with the biotech sector. RSU plans to collaborate intensely with associations of SMEs to encourage innovation and provide support for these activities. Increasing readiness for further EU initiatives as innovation partnerships and joint programming will be a special emphasis of BALTINFECT.
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