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Immunology and Cell Biology

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Department of Immunology and Cell Biology comprises of three research groups. In 2013-2014, the research was focussed to the following topics: immunogenicity studies of oligomeric antigens; development of monoclonal antibodies; studies on bacterial virulence factors (research group of dr. A. Žvirblienė), regulation of gene expression by alternative splicing (research group of dr. A. Kanopka), molecular epidemiology of tuberculosis (research group of dr. P. Stakėnas).

Reconstitution of *G.vaginalis* toxin vaginolysin into artificial membranes: implications for bioanalysis

Vaginolysin (VLY) is a cholesterol-dependent cytolysin, the main virulence factor of bacteria *Gardnerella vaginalis*. In the current study, we have investigated the functional reconstitution of VLY into artificial tethered bilayer membranes (tBLMs) composed of synthetic dioleoylphosphocholine with variable amounts of cholesterol. The reconstitution was followed in a real-time by the electrochemical impedance spectroscopy (EIS). Changes of the EIS parameters of tBLMs upon exposure to VLY solutions were consistent with the formation of water-filled pores in membranes. It was found that reconstitution of VLY is a strictly cholesterol dependent, irreversible process. In the absence of cholesterol no effects on membrane permeability or dielectric properties were detected, while increased effect was observed with increasing cholesterol content in tBLM. At constant cholesterol concentration, reconstitution of VLY occurred in a VLY concentration-dependent manner thus allowing monitoring protein concentration and activity in vitro and opening possibilities for tBLM utilization in bioanalysis. Inactivation of wild-type VLY by amino acid substitutions led to a noticeably lesser tBLM damage. Pre-incubation with the neutralizing monoclonal antibody inactivated the membrane damaging ability of VLY in a concentration-dependent manner, while non-neutralizing antibody exhibited no effect. Interestingly, contrasting earlier findings a membrane-damaging interaction between VLY and tBLM was observed in the absence of a human CD59 receptor, which is known to strongly facilitate hemolytic activity of VLY. Estimates allowed us to conclude that in the absence of the CD59 much smaller amount of pores are formed in cells so that hemolytic activity of VLY is almost suppresed. Taken together, our study demonstrates applicability of tBLMs as a bioanalytical platform for the detection of the activity of VLY, and possibly other cholesterol dependent cytolysins.

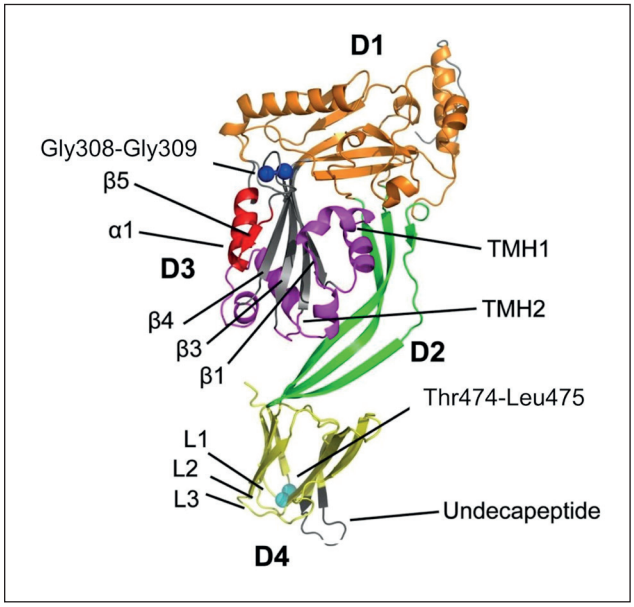

Figure 1. Molecular model of vaginolysin (created by dr. Č. Venclovas). Domains are designated as D1-D4; TMH1-TMH2 motifs—purple ribbons; the double Gly motif is shown as dark blue space-filled spheres; the Thr-Leu pair in D4 is shown as light blue spheres.

This study has been performed in collaboration with the Institute of Biochemistry of Vilnius University.

Reference:

Budvytyte et. al., Plos One, 2013; 8(12):e82536. doi: 10.1371.

Studies on the immunogenicity and cytotoxicity of amyloid beta oligomers

The central molecule in the pathogenesis of Alzheimer's disease (AD) is believed to be a small-sized polypeptide – beta amyloid (Aβ) which has an ability to assemble spontaneously into oligomers. Various studies concerning therapeutic and prophylactic approaches for AD are based on the immunotherapy using antibodies against Aβ. It has been suggested that either active immunization with Aβ or passive immunization with anti-Aβ antibodies might help to prevent or reduce the symptoms of the disease. However, knowledge on the mechanisms of Aβ-induced immune response is rather limited. Previous research on Aβ1-42 oligomers in rat brain

cultures showed that the neurotoxicity of these oligomers considerably depends on their size. In the current study, we evaluated the dependence of immunogenicity of Aβ1-42 oligomers on the size of oligomeric particles and identified the immunodominant epitopes of the oligomers. The analysis of serum antibodies in mice immunized with various Aβ oligomers revealed that small Aβ neurotoxic oligomers (1-2 nm in size) are highly immunogenic. In contrast, larger Aβ oligomers and monomers did not induce a detectable IgG response. Monoclonal antibodies against 1-2 nm Aβ oligomers were generated and used for the antigenic characterization of Aβ oligomers. Epitope mapping demonstrated that the main immunodominant region of the Aβ oligomers is located at its N terminus (aa 1-13) thus indicating its surface localization and accessibility to the B cells.

We have investigated whether monoclonal antibodies to Aβ oligomers would prevent their neurotoxicity in primary neuronal-glial cultures. However, surprisingly, the antibodies dramatically increased the neurotoxicity of Aβ oligomers. Moreover, antibodies to other oligomeric proteins (recombinant virus-like particles) strongly potentiated the neurotoxicity of their target antigens. The neurotoxicity of antibody-antigen complexes was abolished by removal of the Fc region from the antibodies or by removing microglia from cultures. This indicates that that immune complexes formed by Aβ oligomers or other oligomeric antigens and their specific antibodies can cause death and loss of neurons in primary neuronal-glial cultures via Fc-dependent microglial activation.

The results of the current study may be important for further development of Aβ-based vaccination and immunotherapy strategies.

This study has been performed in collaboration with the Institute of Biochemistry of Vilnius University and the Lithuanian University of Health Sciences (Kaunas).

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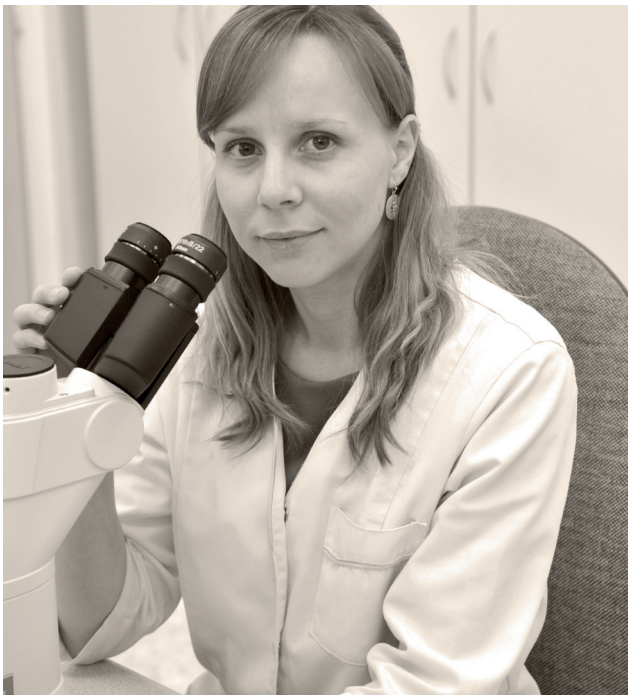
Dalgediene et al., J Biomed Sci 2013, 20:10. doi:10.1186/1423-0127-20-10.

Morkuniene et al., J Neurochem 2013, 126:604-615.

Development and characterization of monoclonal antibodies against cellular and viral antigens

The Department has long-term experience in development and characterization of monoclonal antibodies against various targets. During 2013-2014, a large collection of monoclonal antibodies against viral and cellular proteins has been generated.

Previous studies have shown that recombinant viral structural proteins with their intrinsic capacity to self–assemble to highly-organized structures - virus-like particles (VLPs) or nucleocapsid-like particles (NLPs) – are highly immunogenic and represent promising antigens for developing virus-specific antibodies. In collaboration with the Department of Eukaryote Gene Engineering, novel monoclonal antibodies against recombinant yeast-expressed viral antigens have been generated and employed for diagnostic assays. Those include antibodies against hamster polyomavirus VP1 protein (Munoz et al., Arch Virol. 2013), human parvovirus 4 capsid protein (Tamošiūnas et al., Intervirology 2013), porcine parvovirus capsid protein (Tamošiūnas et al., J Immunol Res 2014) , Schmallenberg virus nucleocapsid protein (Lazutka et al., J Immunol Res 2014) and hantavirus glycoprotein (Zvirbliene et al., Viruses 2014).



Junior scientist R. Lasickienė

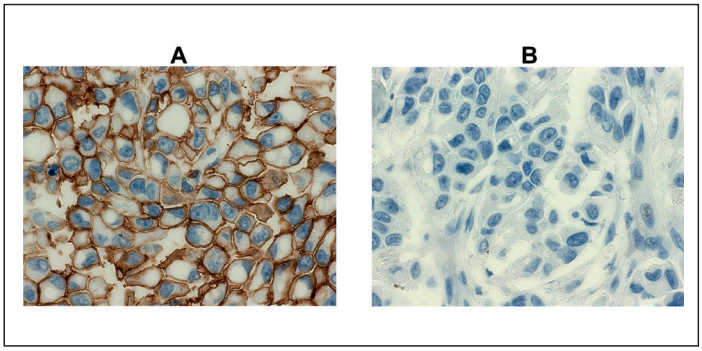


Figure 2. Immunohistochemistry staining of human invasive ductal carcinoma for CA XII expression using the CA XII-specific antibody 3D8 (A). An irrelevant antibody 1F8 is used as a negative control (B). CA XII in cell membrane is shown in brown, cell nucleus is shown in blue.

Recombinant antigens have been used to generate novel monoclonal antibodies against human carbonic anhydrase XII, a potential biomarker of tumor cells (Dekaminaviciute et al., J Enzyme Inhib Med Chem 2014, Dekaminaviciute et al., Biomed Res Int 2014). The diagnostic relevance of the newly developed antibodies has been confirmed using tumor cell lines and clinical specimens (Figure 2).

A large collection of neutralizing monoclonal antibodies against DNA polymerases has been developed (contract with Thermo Fisher Scientific, No AP5-560000-1032).

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Dekaminaviciute et al., J Enzyme Inhib Med Chem 2014, 29(6):804–810.

Dekaminaviciute et al., Biomed Res Int 2014, 2014:309307. doi: 10.1155/2014/309307.

Regulation of hypoxia-dependent alternative pre-mRNA splicing

Hypoxia has been recognized as a common feature of solid tumours and a negative prognostic factor for response to treatment and survival of cancer patients. Biological responses to hypoxia involve induction of transcription of a network of target genes, a process which is co-ordinately regulated by three structurally related hypoxia-inducible transcription factors (HIFs): HIF-1, HIF-2 and HIF-3. HIFs recognize hypoxia response elements of target genes as heterodimeric complexes (HIF-1α, HIF-2α and HIF-3α) with the transcription factor Arnt.

Research group of dr. A. Kanopka: I. Pečiulienė, A. Mazėtytė, K. Vaidžiulytė, dr. A. Kanopka, E. Jakubauskienė, L. Vilys

A striking change has been observed in alternative splicing patterns of genes and alterations in splicing factor expression under pathologic conditions especially in human cancers. Cancer cells are often confronted with a significant reduction in oxygen availability. The splicing machinery heavily contributes to biological complexity especially to the ability of cells to adapt to different developmental stages and altered cellular conditions. The selection of alternative splice sites can be regulated in a different manner related to tissue specificity, developmental stage, physiological processes, sex determination and in response to various stress factors. A number of reports describe changes in alternative pre-mRNA splicing patterns induced by hypoxia. The mechanism underlying oxygen tension-dependent changes in splicing remains unknown.

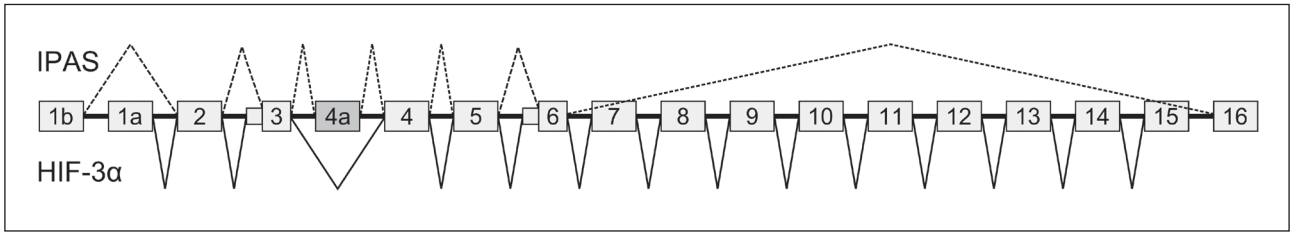

Figure 3. ***Alternative hypoxia-dependent HIF-3α pre-mRNA splicing in mice. HIF-3α mRNA is produced both in normoxic and hypoxic cells. IPAS mRNA is produced strictly in hypoxic cells.***

Our goal is to establish mechanism and factors involved in hypoxia-dependent splicing regulation. We established that hypoxia-inducible factor HIF-1 indirectly is involved in such regulation. Also we established that a change in activity of essential splicing factors determine oxygen-dependent pre-mRNA splicing.

Thus we identified one of hypoxia-dependent pre-mRNA splicing regulator which might re-program cellular events and could not only be useful for the potential therapeutic applications but also for their application as an analytic tool.

This work was supported by the EU Framework 7th Programme (project Metoxia).

Molecular epidemiology of *Mycobacterium tuberculosis*

Tuberculosis (TB) caused by *M. tuberculosis* complex bacteria remains a serious health problem in Lithuania. Incidence of TB and in particular multidrug-resistant (MDR TB) is one of the highest in the European Society. The aim of our study is to characterize population of *M. tuberculosis* strains that circulate in Lithuania including the genetic determinants of drug resistance. The research was carried out in collaboration with Infectious Diseases and Tuberculosis Hospital, Affiliate of Vilnius University Hospital Santariskiu Klinikos and other partners from the research of TB networks. Genotyping was performed by international standardized molecular methods (MIRU-VNTR typing, spoligotyping) and the polymorphisms of *M. tuberculosis* genome were identified by a direct sequencing. The data were submitted to the relevant multinational databases that facilitate understanding of the spread of TB and emergence of drug resistance. Analysis of *M. tuberculosis* genotypes indicated that many of the Lithuanian isolates are in the cross-borders clusters. Therefore, we started sub-typing of the strains by using an additional hypervariable MIRU-VNTR locus in order to improve a discrimination power of MIRU-VNTR typing. Also, we continued the identification of the mutations occurring in the well-known genomic regions of *M. tuberculosis* involved in drug resistance and search for polymorphisms in the putative targets for the first and second line anti-TB drugs. A large multicenter study on a deep characterization of polymorphisms in the pncA gene involved in the resistance to the key drug pyrazinamide was completed and published.

This work was supported by the EU Framework 7th Programme (project TB PAN-NET).

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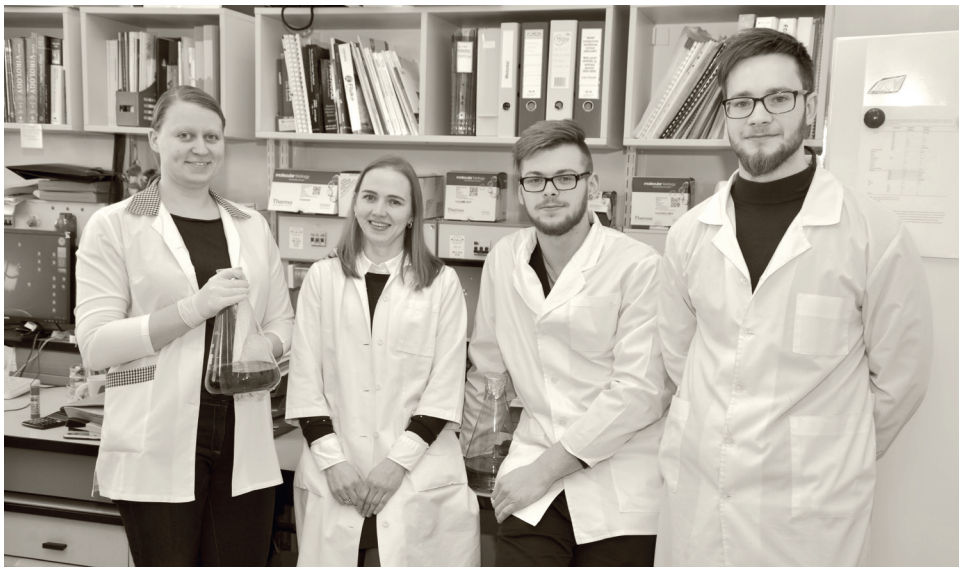

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Publications 2013-2016

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