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REVIEW ARTICLE

INSTITUTE OF MOLECULAR PATHOLOGY, FMHS, UoD, HRADEC KRALOVE – TWENTY YEARS OF EXISTENCE

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Summary

The Institute of Molecular Pathology was founded on May 1, 1994 as a scientific centre of the Purkyne Military Medical Academy. During the twenty years of its existence the Institute has gained reputation of a qualified research centre focused on infection biology, clinical proteomic studies and biodefense. The advanced proteomics combined with cellular and molecular biology techniques and broad international collaboration have enabled the Institute to educate Ph.D. students at the highest possible level. Meetings, workshops, and conferences organized by the Institute have been attended by recognized scientists. The twentieth anniversary of the Institute foundation is a good opportunity to recap the results of its work.

Key words: Institute of Molecular Pathology; Proteomics; Francisella tularensis; Biodefense

INTRODUCTION

The history of the Institute dates back to May 1st, 1994. The reason for its creation was to ensure the continuity of basic microbiological research after staffing and spatial limitations of the military establishment for biological research in Techonin. The original ideas were to create a scientific centre that will have all the attributes of prestigious research institutions abroad. It means that it will have its own research programs based on the advanced technologies, which will be owned by the Institute, it will educate Ph.D. students, and will be an equal partner in international research collaboration. Moreover, it will organize the international meetings and its members will participate in international research groups. The majority of these original ideas have been fulfilled within twenty years of the Institute existence.

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HISTORY

Biological research program of the Czechoslovak Army was opened during the Cold War in the late 1950's. At the beginning, the first experiments were realized in a microbiological laboratory located in Prague, the district Orechovka. This laboratory was a part of the Military Institute of Hygiene, Epidemiology and Microbiology, Prague. Soon after the establishment (in 1961), the laboratory was relocated to a small village Novy Hradek as the Biological Station of the Czechoslovak Academy of Sciences. From the beginning, the complex of laboratories had special security regime and was guarded by armed

guards with dogs. One of them was originally a lion tamer [1]. The military part of the Novy Hradek laboratory complex functioned until 1968. During the years 1968 – 1972 military laboratories were moved into a newly built laboratory complex in Techonin. This military establishment had three departments; the Department of Virology, the Department of Bacteriology, and the Department of Rickettsiology. The dominant scientists were Rudolf Benda (virology), Jiri Libich (bacteriology), and Frantisek Rehn (rickettsiology). The original studies were oriented to the pathogenesis of biological agents predicted to be misused in the form of biological weapons (B-agents). The immunological studies at the cellular level started with the advent of other young scientists (Petr Propper, Jan Kopecký, Ales Macela) in the Techonin labs.

On March 17, 1993, an unsubstantiated affair with the storage of biological weapons in Czechoslovakia arose, which together with the reduced budget of the Ministry of Defence, was the signal for reflection on the future of this military base for biological research. Finally, in January 1994 it was decided to limit strictly both the research activities oriented to inhalation induced infections by B-agents and the number of personnel engaged in biological research in the Czechoslovak Army. As a counterbalance to this step, it was decided to create a new institute that will be subordinate to Purkyne Military Medical Academy located in Hradec Kralove. So, the Institute of Immunology, recently Institute of Molecular Pathology, came into existence on May 1, 1994.

RESEARCH

The original idea was to create the basis for basic research on B-agents realized on advanced technologies and,at the same time, to educate Ph.D. students at the highest possible level and to prepare them for work in other departments of the Academy or in military units designed to ensure biosecurity. The proteomic technologies, chosen as a leading technology for molecular analyses, have been combined with the methods of cellular and molecular biology, and imaging techniques.

The scientific team of the Institute was compiled depending on the focus of the science. Jiri Stulik, Hana Kovarova and two technicians Jana Michalickova and Alena Firychova ensured the molecular analyses, Lenka Hernychova, Zuzana Krocova, Michal Kroca, and Ales Macela with the technicians Radka Krejcova and Zdenek Safek realized the microbiological and immunological part of studies. The program of scientific centres creation financed by the Czech Grant Agency enabled the origin of the Proteomic Centre for the Study of Intracellular Parasitism (2000-2004) at the Institute and attracted young scientists. After finishing this program, the science at the Institute was extended by application of proteomic techniques to clinical studies (Juraj Lenco), and defence and security programs (Martin Hubalek). Thus, scientific profile of the Institute was stabilized during the years in three main branches; host-pathogen interaction at the model of *Francisella tularensis* (*F. tularensis*), defence and security studies, and clinical studies with the utilization of proteomic techniques.

Host-Pathogen Interaction Analyses

Host-pathogen interaction was studied from both the site of the host and from the site of bacterium. At first, the genetic background of immune responsiveness to *F. tularensis* infection [2–4] was studied. At the cellular level, the interaction of *F. tularensis* with macrophages [5], B cells [6,7], and dendritic cells [8] was studied. These studies demonstrated that infection of cells with *F. tularensis* strains causes the expression of host cell stress proteins, activates MAPK signalling pathways, and orientates the infected cells to apoptosis [9]. The study of the early stages of cell infection provides the evi-dence that infection of macrophages with *F. tularensis* leads to changes in protein composition of macrophage-derived lipid rafts, activation of au-tophagic pathway, and, that by such a way the intracellular trafficking of the invading bacterium [10,11] is determined. On the other side, a global proteome analysis of a virulent *F. tularensis* during its intracellular cycle within the macrophage-like murine cell line J774.2 using the metabolic pulse-labelling of bacterial proteins demonstrated that *Francisella* has the ability to adapt to intracellular hostile environment [12].

Experience with the high resolution two-dimensional gel electrophoretic procedures, Western blot technique, and mass spectrometric analyses enables us to map *F. tularensis* global proteome, secretome, and membranome [13–16], to disclose the differences in protein composition of three *F. tularensis* subspecies [17,18], to identify immunoreactive *F. tularensis* proteins [19,20],

and to study the response of bacteria into the artificial stress conditions [21,22].

The complete sequencing of *F. tularensis* genome by consortium of scientists from Sweden, the UK, and the US [23] has a profound impact on the study of subcellular localization and function of individual proteins. It should be noted that *F. tularensis* genome codes a number of hypothetical proteins that have no homologs in proteins of other prokaryotes. Moreover, the question of molecular basis of *Francisella* virulence was still open. Thus, the proteome technology seemed to be an ideal tool to unravel this question. Step by step, the scientists of the Institute in collaboration with scientists from France and Sweden demonstrated that ClpB heat-shock protein is involved in stress tolerance and is required for *Francisella* intracellular proliferation [24], MoxR family member was identified as a novel player in bacterial virulence [25]. The IglH protein, the gene *iglH* of which is located in *Francisella* pathogenicity island, was shown to be necessary for intracellular growth and escape of *Francisella* from phagosomes [26] and with *F. tularensis* DsbA homologue constitutes effective virulence factors [27].

Clinical proteomics

The analytical potential of proteomics was utilized for disclosure of changes in cells and tissues of patients that are induced during the onset of the illness. At the beginning, the initial clinical analyses were realized using high-resolution two-dimensional gel electrophoresis only. Combined high-resolution two-dimensional polyacrylamide gel electrophoresis with immunoblotting was used to study hsp70 expression in normal, preneoplastic and neoplastic colonic mucosa [28]. In subsequent studies, the combination of microsequencing and mass spectrometry was utilized to identify the 13 kDa calgranulin B, expression of which demonstrated to be unregulated in inflammatory, preneoplastic and neoplastic lesions of colonic mucosa [29]. More detailed mass spectrometric analyses demonstrated the overexpression of calcium-binding proteins S100A8 and S100A9 in colorectal carcinoma. The immunohistological analysis revealed the accumulation of S100A9 positive cells, macrophages and polymorphonuclear leukocytes, along the invasive margin of colorectal carcinoma [30]. The following study demonstrated that the levels of liver fatty acid-binding protein, actin-binding protein/smooth muscle protein 22-alpha and cyclooxygenase 2 were down-regulated in colorectal carcinoma compared to normal colon mucosa. Conversely, the expression of the novel variant of heat shock protein70 and several members of the S100 protein family of calcium-binding proteins (two isoforms of S100A9, S100A8, S100A11 and S100A6) were upregulated in transformed colon mucosa [31].

The realized studies on colorectal tumorigenesis together with other studies thus confirmed that the proteomic approach is useful for the study of complex biological events representing functional status of the tissue and can disclose and identify proteins of importance for diagnosis [32,33].

The new millennium has brought new impetus to the field of clinical proteomics. The studies were oriented to the biomarker discovery of cardiomyopathy [34] and finally to preterm birth. It was documented in this field that the levels of proteins such as soluble scavenger receptor for haemoglobin (sCD163), haemoprotein myeloperoxidase, or cathelicidin, and antimicrobial peptide, can be candidate markers facilitating the identification of pregnant women at risk of preterm birth [35–38].

Defence and security studies

The defence and security studies were realized in association with the Czech Army biodefense programs, the European Defence Agency (EDA), or NATO Research and Technology Organization. In general, the studies were realized in two areas of concern. The results from molecular analyses of microbes, as *F. tularensis*, *C. burnetii* or *Burkholderia malei* were utilized for the development of molecular markers for detection, identification, and verification of biological threats. In parallel, the results from global proteomic analyses were the basis for the application of genomic and molecular biology methods with the aim to give basic data utilizable for creation of a safe and effective live vaccine in future. All obtained data were collected in the database of the European Biological Laboratory Network interconnecting the biological labs from eleven European countries (EDA project). Some studies on animal models were oriented to the problems of immediate prophylaxis of infections in the case of real combined CBRN incident [39].

The theoretical branch of defence and security research has been devoted to the issues of biological crises management; to define the basic parameters

needed for risk assessment, to prepare real scenarios or vignettes of biological incidents, and to give recommendations to the state administration. In this respect, the Institute has closely collaborated with partners from EU and the US.

The Institute of Molecular Pathology was an active and important participant in international activities focused on defence and security. The Institute organized NATO Task Group meetings and significant conferences on the problems of CBRN security or Dual-use technologies in Hradec Kralove. The great success was achieved by the International Workshop on Biological Crisis Management held in Hradec Kralove in 2008. The studies realized under the COST Action program were issued in two books, "Detection of Highly Dangerous Pathogens" and "BSL3 and BSL4 Agents, Epidemiology, Microbiology, and Practical Guidelines", with active participation of the scientists from the Institute. The knowledge, skills and new ideas of the Institute personnel have been also presented at the meetings and conferences dedicated to the biosecurity in Germany (Munich, Munster), UK (Porton Down), the Netherlands (Rijswijk), Sweden (Umea, Kista), in Brussels, in the US (Fort Detrick), and so on.

EDUCATION

The advanced laboratory equipment and the skilled scientific team enabled the accreditation of a Ph.D. study program. At first, it was the program called "Molecular Pathology", nevertheless, it was not as attractive for students as we expected. Moreover, there were problems with a clinical part of the program. The interest of students was rather focused on proteomics applied on problems of host-pathogen interaction. Therefore the new program entitled "Infection Biology" was accredited in 2006. Since then, the Institute has educated students who get positions at civilian universities, in the Czech Academy of Sciences, and who carry out research in scientific centres abroad, for example in Sweden or in the UK. Some graduates of this study program take up an appointment in the Institute itself. The scientific team of the Institute gives lectures on proteomics at faculties of Charles University located in Hradec Kralove and other universities in the Czech Republic. Thus, the Institute has become one of the effective promoters of proteomics in the Czech Republic.

CONCLUSION

The Institute of Molecular Pathology has left behind significant traces in the field of infection biology and proteomics within twenty years of its existence. The members of the Institute published articles that are frequently cited. The conferences organized by the Institute alone or in collaboration with scientific societies of the Czech Republic attracted outstanding scientists from European countries and the US as well. The conferences "Experimental, Therapeutic and Toxic Manipulation of the Host Immune System" and „International Conference on 2-D Electrophoreses" held in 1995 allow the involvement of scientists of the Institute in the world scientific community. Organization of "The Second International Conference on Tularaemia" entrusted to the Institute confirmed its high reputation in the scientific community. The same can be said about the "Host-Pathogen Interaction Forum", organized since 2001, which has been recently the international platform for exchange of knowledge and ideas on infection biology.

Thus, the Institute of Molecular Pathology is a scientific centre oriented towards the analyses of living systems at the level of tissues, cells, proteins, and nucleic acids which is fully integrated into the network of world scientific centres. The established international collaboration ensures continuous methodological development and, in parallel, the objectification of research results.

Finally, let us hope that the Institute has still many years of its existence ahead and it will retain the level of science that it has achieved till now.

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