Four decades of immunology in Croatia: University of Rijeka, Medical Faculty

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Development of immunology in Rijeka is entirely connected with the development of the Medical Faculty in Rijeka which was established 52 year ago (in 1955). This was first higher education institution in Croatia that was founded outside Zagreb. Today, it is the largest scientific and teaching higher education institution outside Zagreb incorporated into the University of Rijeka since 1973. It has more than 450 teaching staff, several undergraduate, graduate and postgraduate training programs, and distinguished international scientific reputation. Immunology, as one of first scientific disciplines developed at the new Medial Faculty, contributed substantially to this reputation.

In this paper we will give a brief overview of the last 45 years of development of immunology in Rijeka that started with the establishment of the Department of Physiology and disseminated towards a number of Medical Faculty departments and health system institutions in the city of Rijeka. It will not be a strict historical listing of major events, but rather description of dissemination of immunology with development of major actors, researchers and physicians, and their achievements over four decades. At the end of the article, we believe that the reader will recognise the power of Rijeka's immunological community and huge immunological expertise that lays in this part of Croatia.

DEPARTMENT OF PHYSIOLOGY AND IMMUNOLOGY

The foundation of the Medical Faculty in Rijeka provided the conditions for the development of basic medical sciences and for scientific and highly professional enrichment of the overall medical activities in Rijeka. However, the origins of immunology in Rijeka date back to 1964, when Professor Nikša Allegretti was for a one year period elected acting head of the Department of Physiology. Professor Daniel Rukavina (since February 1962) and Professor Predrag Eberhardt (since September 1963) had already been his collaborators at the Department.

Subsequently, thanks to N. Allegretti, in September 1965, head of the Department of Physiology became Šime Vlahović (Figure 1). Šime Vlahović had acquired his basic scientific education at the Ruđer Bošković Institute and then stayed in the USA for three years. He worked in Cooperstown (1963–1965) and in Boston (1965) on the problems of bone marrow transplantation to irradiated adult recipients (mice and dogs) exposed to lethal and sublethal irradiation doses, and on selection of the most suitable transplant donor. It later turned out that his research interest and education were of extraordinary significance for the development of immunology and clinical transplantation in Rijeka.

Figure 1. ***Dr. Šime Vlahović.*** *First head of the Department of Physiology and Immunology (1965–1977).*

At that time, several groups of excellent surgeons worked at the Medical Faculty and the Clinical Hospital, of which the group led by Professor Vinko Frančišković was exceptionally interested in new approaches to therapy of irrecoverably lost kidney function (chronic dialysis and transplantation). With the arrival of Š. Vlahović at the position of the Department head, research interest was focused in several areas: (1) study of conditions to prolong the life of tissue transplants, (2) immunology of the mother/foetus relationship, and (3) the role of lymphatic system in control of the rapid growth of normal tissue (compensatory growth after unilateral nephrectomy and partial hepatectomy). Soon afterwards, in October 1966, Biserka Radošević-Stašić became an employee of the Department and took active part in dealing with this problem area. After some time she also established her own research group that was later joined by Mira Ćuk. A great contribution in the field of immunoregulation gave also Vlasta Linić-Vlahović, who jointed to the Department. In 1972 D. Rukavina obtained a one year Fulbright postdoctoral fellowship to work at the University of Texas, South-Western Medical School in the group of Professor Rupert Billingham, one of leading scientists in the field of transplantation at that time. He was granted also by special travel grant to visit ten departments of his preference in the field of immunology and transplantation which helped him to establish professional and personal contacts with many leading scientists in the field.

After a too early demise of Š. Vlahović (March 9, 1977, in his 45th year), D. Rukavina became head of the Department. In the same year, the Department underwent the first substantial functional and architectural reconstruction and three laboratories for research in the field of cellular immunology were established. Thus, lymphocyte subpopulations have been determined since 1977 in Rijeka using the techniques of forming rosettes with xenogeneic red blood cells, serum immunoglobulins have been determined in serum and secretions by immunochemical methods, and functions of lymphocyte reactivity on the panel of polyclonal mitogens, specific antigens and alloantigens were investigated. Determination of alloreactivity in cultured mixed lymphocyte reaction (MLR) was of particular interest also because of its application in clinical transplantation.

In 1979, the Department of Physiology and Immunology became the teaching base also for the course in Pathologic Physiology. B. Radošević-Stašić took up the leadership of this course. All these events in the 1978–1980 period allowed employment of a large group of young collaborators who distinguished themselves during studies as best students, worked at the Department as undergraduate assistants and took part in research activities. An atmosphere filled with extraordinary enthusiasm and the wish to perform new breakthroughs in science was established. We can especially point out Miljenko Dorić, Stipan Jonjić, Miro Morović and Miljenko Kapović in that group (Figure 3).

Figure 2. ***Teaching staff at the Department of Physiology and Immunology in 1977.*** *From the left: Miro Morović, Predrag Eberhardt, Daniel Rukavina, Biserka Radošević-Stašić and Mira Ćuk.*

In 1986, a major reconstruction was carried out at the Department of Physiology and Immunology including very modern furnishing and equipment of research laboratories, rooms for experimental animals, and rooms for classes. In the following ten years a number of cellular and molecular immunology techniques was introduced and expanded, including the cell culture, hybridoma technology and maintenance of cell lines. This was followed by extensive production, purification, characterization and usage of monoclonal antibodies, particularly through flow cytometry, various enzyme linked immunoassays, Western blotting and immunoprecipitation. In the decade 1995–2005, a number of modern molecular biology techniques were introduces.

During two decades of intensive growth (1977–1998), more than ten young collaborators were employed at the Department during one to eight years who, after acquiring academic degrees and basic scientific education under the supervision of D. Rukavina and B. Radošević-Stašić, transferred to many other departments of the Medical Faculty. Today, 22 researchers are employed at the Department, of which 12 have scientific-teaching position and 10 assistants.

Figure 3. ***Members of the Department of Physiology and Immunology in 1981.*** *Sitting from the left: Biserka Radošević-Stašić, Vlasta Linić-Vlahović, Antonija Peršić, Daniel Rukavina, Dragica Kovačević, and Predrag Eberhardt. Standing from the left: Stipan Jonjić, Mira Ćuk, Miljenko Kapović, Marija Kaštela, Jelena Đirlić, Davorka Perčinić, Nadija Peraić, Miljenko Dorić, and Vjera Vučenov.*

Through organized choice of collaborators and research problem areas and through education of collaborators in top-level European laboratories, an atmosphere for further strengthening of immunology in Rijeka was created. Thus M. Dorić stayed on several occasions in Paris, S. Jonjić in Tübingen and Ulm, M. Kapović in Paris, P. Lučin in Ulm and Heidelberg, Vesna Barac-Latas in Wienna, Damir Muhvić in Borstel-Lübeck and Zlatko Trobonjača in Ulm. Researchers and collaborators of the Department have published more than 250 papers in international journals indexed in the Current Contents database. They maintain close collaboration and contacts with a number of European and American research groups.

The expansion of research activities at the Department dates back to 1977, when first projects of the former Republic Scientific Fund were allocated to D. Rukavina. After that, the number of organized research activities was constantly increasing, including national and international grants. This resulted with the inclusion of the group of researchers from Department, leading by D. Rukavina into the Framework Programme 6 network of excellence EMBIC (Embryo Implantation Control – Understanding the molecular tuning of the beginning of life).This network is the first large-scale project funded by the European Commission within the FP6 to reinforce European research on female infertility. It concentrates research potential of more than 200 scientists and clinicians from 19 leading European institutions and 2 private companies from 11 countries. D. Rukavina was one of the initiators of the network and the first meeting of the initial group of nine laboratories to define the project proposal was held in March 2002 in Rijeka *(1)*.

During last four decades, the work in laboratories of the Department resulted with more than 90 Doctoral and Master of Science theses. This means that a number of researchers and teachers of the Medical Faculty and Clinical Hospital Centre of Rijeka acquired their basic scientific competences at the Department.

In addition to their research and teaching activities and achievements, the entrepreneurial and free academic spirit at the Department contributed also to the development of leadership and management competencies of teaching staff of the Department. These competences significantly contributed to the development of the Medical Faculty, University of Rijeka and national policies. D. Rukavina, S. Jonjić and M. Kapović were elected Deans of the Medical Faculty, and D. Rukavina, B. Radošević-Stašić, P. Lučin and V. Barac-Latas Vice-Deans in several mandates. Since 2000, D. Rukavina is the Rector and P. Lučin Vice-Rector of the University of Rijeka. P. Lučin is the first President of the National Foundation for Science, Higher Education and Technological Development of the Republic of Croatia, and Croatian accession negotiator with the EU for chapters Education, Culture and Science. In addition to that, members of the Department chaired a number of committees at the institutional, university and national level (M. Ćuk, Z. Trobonjača, J. Ravlić-Gulan, G. Laškarin). S. Jonjić was the president and P. Lučin vice-president of the Croatian Immunological Society. D. Rukavina was the first president of the re-established Croatian Physiological Society (1999), the first president of the European Federation of Immunology and Reproduction (EFIR), and president of world organization of reproductive immunologists (International Society for Immunology of Reproduction) in the period 2004–2007.

BROADENING OF THE COLLABORATION TO OTHER DEPARTMENTS, CLINICS AND FOREIGN INSTITUTIONS

Such development of research potential at the Department of Physiology and Immunology was exceptionally stimulating for the advancement of general research atmosphere at the Medical Faculty and for the establishment of research cores also on other departments and clinics. Medical Faculty in Rijeka resolved many personnel problems by selecting collaborators who attained basic scientific education at the Department of Physiology and Immunology. Thus, M. Dorić became head of the Department of Microbiology and Parasitology in 1987, M. Kapović of the Department in Biology in 1991, S. Jonjić of the Department of Histology and Embryology in 1996, and M. Morović led the Clinics of Infective Diseases. Immunological studies developed then in these basic departments, as well as in the Department of Pathology where Nives Jonjić was elected head in 1993, while Marija Petković became head of the Department of Oncology. A significant number of clinicians who got their basic scientific education and acquired academic degrees at the Department of Physiology and Immunology were in the past period or are presently heads of departments or division heads in clinics: Nikola Matejčić, Herman Haller, Oleg Petrović, Darko Manestar, Darko Ledić, Luka Zaputović, Ksenija Vujaklija-Stipanović, Sanja Balen-Marunić, Anđelka Radojčić-Badovinac, Jovan Tofoski, and Vladimir Vincek.

Collaboration with numerous institutions and eminent researchers from abroad is very extensive and has yielded significant results, which was evident in continuous education of young scientists in foreign institutions, in numerous publications in famous world journals and abundant help in material and equipment. Thus, collaboration was established with laboratories in Paris (G. A. Voisin and G. Chaouat), Chicago (A. E. Beer and K. Beaman), Pittsburgh (T. J. Gill III), Miami (E. R. Podack), Ulm and Tübingen (U. Koszinowski, T. Mertens, J. Thiele, M. J. Reddehase), Borstel-Lübeck (H. D. Flad), Vienna (H. Lassmann), Stockholm (S. Efendić), Pecs (J. Szekeres-Bartho), Liverpool (P. Johnson and S. Christmas), and Milano (A. Mantovani).

DISSEMINATION OF IMMUNOLOGY TO THE DEPARTMENT OF HISTOLOGY AND EMBRYOLOGY

The group of immunologists currently affiliated at the Department of Histology and Embryology originally started their research at the Department of Physiology and Immunology. In 1996 S. Jonjić and part of his group moved to the Department of Histology and Embryology and continued their research work in immunology and experimental pathology. In addition to Bojan Polić and Astrid Krmpotić, several PhD students also joined them and the group continued their research without interruption. The Department was completely renewed and several fully equipped new laboratories were established. Two scientists already affiliated at the Department also joined this group – Ester Pernjak-Pugel and Jelena Tomac.

The group was growing from year to year and through several international and national research projects very high scientific standard was reached, with several publications in leading journals in the field of immunology and virology. Thanks to this, B. Polić was awarded Humboldt Fellowship (1997–1999) and spent his postdoctoral studies in the group of Klaus Rajewsky in Cologne. After finishing her PhD Milena Hasan received EMBO Fellowship and did her postdoctoral studies in Pasteur Institute in Paris and Luka Čičin-Šain spent several years in Germany working in the group of Ulrich Koszinowski. Both of them later on decided to continue their careers abroad. In 2006, A. Krmpotić was awarded Howard Hughes Medical Institute International Research Scholar Grant to perform the research in her home laboratory.

Throughout these years research groups at the Department developed collaborative research activities with many laboratories abroad including Ulrich Koszinowski (Max von Pettenkofer-Institute, München), William J. Britt (UAB, Birmingham), Mathias Müller, (Veterinary University of Vienna), Hartmut Hengel, (Heinrich-Heine-University Düsseldorf), Martin Messerle (University of Halle-Wittenberg), Wayne M. Yokoyama (Washington University Medical Centre, St. Louis), Joanne Trgovcich (Ohio State University, Columbus), and Silvia Vidal (McGill University, Montreal).

IMMUNOLOGICAL RESEARCH AT THE DEPARTMENT OF MICROBIOLOGY AND PARASITOLOGY

Immunological research diffused to the Department of Microbiology and Parasitology when Miljenko Dorić moved to this department in 1987. He has a long standing interest in scientific research on the pathogenesis of bacterial infections. At the beginning the research was primarily focused in new approaches of bacterial identification by the assistance of the numerical-computer system (M. Dorić and Janko Makiš) and in 1997 Dorić introduced a model of experimental murine congenital listeriosis. Almost simultaneously, Maja Abram established a murine model of pregnancy-associated listeriosis, Darinka Vučković *in vivo* model of *Campylobacter jejuni* infection, Tomislav Rukavina started model of *Klebsiella* infection and Brigita Tićac model of *Legionella pneumophila*. The Legionella team was extended in the period by two new researchers, Marina Šantić (arrived in 1998) and Ivana Gobin (2001), while Marina Bubonja who came in 2000 joined the research concerning listeriosis.

In the period 2002–2006 scientific research at Department was extended, as two researchers (M. Abram and T. Rukavina) became independent, leading their own projects. Subsequently in 2006, three other researchers were able to submit their projects and establish their own research teams. In the new project period started in 2007, M. Dorić is coordinating the research program composed of 6 research projects, which is focused on different pathogens that are associated with infectious diseases of significant public health impact: legionellosis, listeriosis, tularemia, campylobacteriosis and gram-negative sepsis. Besides studying the properties of pathogens, the research groups also analyze the host's susceptibility to infection and the ability of the host immune system to control and eliminate the microorganisms. The program is realized in collaboration with researchers from Ljubljana (B. Wraber, S. Smole-Možina, B. Jeršek), Louisville (Y. Abu Kwaik), Cologne (M. Deckert), and Stuttgart (M. Šuša).

IMMUNOLOGY AT THE DEPARTMENT OF PATHOLOGY

Although a majority of research focuses of the Department of Pathology do not fit into the field of immunology, it is important to note that immunological techniques are in the basis of the routine diagnostic performed by the Department. These techniques were introduced since 1993, when Nives Jonjić became head of the Department. In the first period the imunnohistochemistry was performed on frozen sections and couple of years later extended to termostabile epitopes on paraffin embedded tissue sections. Today, the Department is characterizing a panel of more than 200 tissue antigens in phenotyping of tissue histogenesis, particularly in tumour diagnostics. Lymphoma phenotyping was introduced in 1996 and since then the pathologists from the Department are following trends in modern diagnostics of lymphoma, the revised classification from 1997 and the WHO classification from 2000. The determination of tumour histogenesis, particularly predictive diagnostics of tumour markers and receptors is exponentially growing, including also recognition and competencies of Rijeka's pathologists.

In addition to immunihistochemistry, immunofluorescent techniques in diagnostics of autoimmune diseases and transplant rejection were introduced since 1993. It can be expected that also immunogold electron microscopy will be introduced in the close future, given that the complete infrastructure was established in the last five years.

CLINICAL IMMUNOLOGY AND TRANSPLANTATION IN RIJEKA

Development of basic immunologic research at the Department of Physiology by Š. Vlahović in 1965 could not pass unnoticed so that clinicians expressed their interest in collaboration. The core transplantation group was soon set up consisting of clinicians headed by V. Frančišković and scientists from basic research institutes led by Š. Vlahović (D. Rukavina, V. Linić-Vlahović, P. Eberhardt, and B. Radošević-Stašić). Regular meetings were held (27 meetings in total) where current aspects of immunology and transplantation and clinical experiences were discussed. Young clinicians went to other centres to acquire education (Petar Orlić, Ksenija Vujaklija). All that resulted in quality preparations for the first successful kidney transplantation in 1971, not only in Croatia but also in the former state and even in southern Europe (Figure 4). It was an extraordinary impetus to development of clinical medicine in Rijeka, as well as to progression of immunological studies.

This stage of development could be considered extremely significant for development of immunology in Rijeka. Although we cannot speak of top-level scientific results, still an atmosphere was created of enthusiasm and general support to the development of immunology in Rijeka. Kidney transplantation has become a routine method in the treatment of patients on dialysis so that over 650 transplantations have been performed so far, and Rijeka has remained the leading centre in kidney transplantation. Regrettably, the development of clinical transplantation did not proceed at the pace that it could take so that the transplantation of pancreas took place not before 1994 (P. Orlić, M. Zelić, K. Vujaklija), and the liver in 2006 (M. Uravić).

Figure 4. ***First transplantation team in Rijeka (1971).*** *Gianpaolo Velčić, Miomir Zelić, Damir Dimec, Nikola Gržaja, Daniel Rukavina, Anton Šepić, Alemka Suzanić, Vjerislav Peterković, Duje Vukas, Marija Rakidžija, Branimir Budisavljević, Andrej Gudović, Petar Orlić, Ksenija Vujaklija-Stipanović, Šime Vlahović, Vinko Frančišković, Tomislav Tićac and Jerko Zec.*

TRANSFUSION MEDICINE IN RIJEKA

Development of transfusion medicine was closely linked to the development of immunology and transplantation medicine in Rijeka. In 1956 routine determination of blood groups and Rh factor and in 1964 the indirect Coombs test was introduced. K. Vujaklija established transfusion Centre in 1968 that was transformed into Laboratory for tissue typing in 1971, alongside with the development of clinical transplantation. In 1987, the laboratory was transformed into Department of Transfusiology of the Clinical Hospital Centre (K. Vujaklija-Stipanović, Chairman). Department now employs 10 physicians, 9 laboratory engineers, 29 technicians and 10 accessory staff. Head of Department is Sanja Balen who obtained basic immunology education and PhD at the Department of Physiology and Immunology.

CENTER FOR PROTEOMICS

The Center for Proteomics was established at the Medical Faculty, University of Rijeka in 2006 with focus on monoclonal antibody (mAb) development for cutting-edge applications including proteome analysis. The Medical Faculty made major investments to bring the Center for Proteomics into life. The University allocated financial resources for the building construction, which was completed in summer 2003. The necessary equipment and laboratory furniture was purchased through the Jezgra 17 Grant, awarded by the Croatian Ministry of Science, Education and Sports. Since its start, the basic technology for protein expression and high-throughput mAb production at the Center was organized and supported by the EU FP6 SSA3 Grant. As a first project, generation of mAbs against the entire proteome of the Varicella Zoster Virus (VZV), which consists of 69 distinct proteins, has been performed. Up to now, mAbs have been generated to the vast majority of the VZV proteins. Apart from that, using this comprehensive pool of mAbs as well as other mAbs produced within the Center, the platform for a variety of mAb-based tools for protein analysis will be developed, which should increase the throughput and reliability of proteomic discovery.

In addition to the project mentioned above, the Center for Proteomics is already well integrated into international scientific community. These collaborations are mainly based on the production and characterization of mAbs for research purposes *(2)*. On top of that, the Center also collaborates with several biotech enterprises on the protein expression and the production of mAbs. The Center is taking part in the EU FP6 consortium on the production of various binders to human proteins (Bojan Polic, co- PI).

In collaboration with Dr. Joanne Trgovcich, Ohio State University, Columbus in frame of project supported by Unity through Knowledge Fund the mouse model is used to characterize the entire CMV transcriptome. Altogether, transcriptome research will expand the technological capacity of the Center for upgrading a comprehensive research facility. In the project approved by the National Foundation for Science, Higher Education and Technological Development of the Republic of Croatia a recombinant fusion protein composed of the ligand for the NKG2D receptor and the variable region of the immunoglobulins specific for the viral protein expressed on the surface of infected cells will be generated. It is predicted that the recombinant protein should have the ability to bypass the components of the innate (NK cells) and specific (antibodies) immune responses. If the results are as expected, this will be the first use of mAb-based experimental immunotherapeutic approach designed at the Center.

The Center is engaged in several other ongoing projects, *e.g.* generation of mAbs to ribosomal proteins and several proteins expressed on human NK cells, viral MHC I like proteins for studies in structural biology, potential markers on pancreatic tumour as well as neuro-stem cells and others.

ANIMAL FACILITIES

The greatest obstacle to further progress of not only immunological but biomedical research in Rijeka was the lack of adequate space for cultivation of a laboratory with rodents. Therefore it was necessary to tackle the construction of a special building of vivarium on the grounds of the Medical Faculty. D. Rukavina was active in the realization of this idea and the concept of a modern vivarium in the period 1987–1991, with the support of the Medical Faculty management. Funds for this investment were partly provided by the Ministry of Science, and the remaining funds were made available by the Medical Faculty.

S. Jonjić has taken up the management of the Vivarium since its opening. In time, more than 50 conventional and transgenic mouse strains have been collected and bred in the facility and thus, it became the biggest resource of laboratory mice in Croatia. However, considering the scientific development at the Medical faculty, particularly at the end of 90ties, and new standards in breeding and maintenance of laboratory mice, reconstruction of the facility became a necessity which was recognized by S. Jonjić, who was at the time dean of the Medical School (1999–2003). Therefore, he and B. Polić have designed and reconstructed the Vivarium (2001–2003) in the most modern manner and according to the accepted international SPF standards including strict barrier conditions, IVC systems and quarterly health monitoring. This reconstruction was largely supported by the University of Rijeka and Ministry of Science.

The new Vivarium also contains Laboratory for microinjection and embryo transfer, which is a part of mouse gene targeting program. In 2003 the name of the Vivarum was changed in the Laboratory Mouse Breeding and Engineering Centre Rijeka (LAMRI) and, since than, the management of LAMRI has been taken up by B. Polić. LAMRI is today the most modern laboratory mouse breeding facility in Croatia and represents an important foothold for long-term development of basic biomedical sciences studies in Rijeka and in Croatia.

TEACHING IMMUNOLOGY AT THE MEDICAL FACULTY IN RIJEKA

Teaching of Physiology at the Medial Faculty of Rijeka, led by Professor of the Zagreb Medical Faculty Ljubomir Božović started in the academic year 1957/58. Due to endeavours of Š. Vlahović, the contents of modern immunology were from 1965 gradually included in the teaching of Physiology, and in 1976 a catalogue of competences to be acquired by every student was defined for the first time. V. Linić-Vlahović together with Š. Vlahović was the author of our first textbook in immunology (Bases of Medical Immunology, Otokar Keršovani Publisher, Rijeka 1977). Since 1979, immunology has become the constituent of the course in Physiology (Physiology and Immunology), and the Department of Physiology was in the same year renamed into Department of Physiology and Immunology, with the Chair of Physiology, Immunology and Pathological Physiology active within it.

In the academic year 1992/1993, Immunology disassociated itself as a distinct course and the exam in this course is taken apart from that in Physiology. Comprehensive two-year scientific postgraduate study in Clinical Immunology was founded at the Medical Faculty in 1984 (led by D. Rukavina), with enrolment of a new generation of students every second year. In the 1984, under the supervision of the Dean (D. Rukavina), the first reform of postgraduate studies was performed. Postgraduate

studies in Clinical Pathophysiology with eight different programs were introduced. Comprehensive two year scientific program in Clinical Immunology and Transplantation was established (led by D. Rukavina) with two modules: Clinical Immunology and Experimental and Clinical Transplantation. Establishment of postgraduate studies with lecturers from Rijeka, Zagreb and a substantial number from abroad represented logically rounded off comprehensive activities to ensure a broad personnel basis that was in the near future to even more strengthen the prestige of immunology in Rijeka.

In the 1994/95, postgraduate studies at the Medical Faculty of Rijeka underwent the comprehensive reform, a project led by P. Lučin and S. Jonjić, who was Vice-Rector of the University of Rijeka for science at that time *(3)*. Flexible postgraduate study program Biomedicine, managed for five years by P. Lučin, was created with a number of elective courses in immunology. Former postgraduate Master of Science study program in Clinical Immunology was incorporated in the program of Biomedicine as an elective package of courses led by D. Rukavina since 2000. Seven generations of students were enrolled into postgraduate clinical immunology training during 15 years.

Postgraduate study of Biomedicine was renewed again in 2001 and 2005, and the Clinical Immunology as elective course disappeared from the program. In addition, a number of specialized immunology courses are organized within the new postgraduate program Medical Chemistry, established in 2008 at the level of the University of Rijeka.

ORGANIZATION OF IMMUNOLOGICAL MEETINGS

Numerous national and international meetings were organized by immunologists from Rijeka and they contributed to recognition of Rijeka and Croatian immunology, bringing hundreds of top-level scientists worldwide to Croatia. These meetings allowed and facilitated the establishment of close personal contacts and collaboration and helped in finding bursaries and support for education of young researchers abroad.

First conference of immunologists from the former state with 650 participants was organized in 1985 (Opatija) by immunologists from Rijeka and D. Rukavina was the President of the Organizing Committee.

Dragan Dekaris and D. Rukavina in collaboration with J. Sepčić and M. Dorić and with a support of Croatian Immunological Society organized First Alps Adria Immunology and Allergology Meeting (Opatija, October 1990). The meeting was held under the auspices of Dr. Franjo Tuđman, President of the Republic of Croatia. The meeting was attended by remarkable number of leading immunologists (350 participants) from Alps Adria region and other parts of Europe and USA *(4)*.

The war against Croatia prevented the organization of the second meeting which was planned for 1992. In the meantime the profile of the meeting was changed and the official names of the meetings were »Mechanisms in Local Immunity« that were held in 1994, 1996 and 1998. The organizers of these meetings were D. Rukavina and Thomas J. Gill. Presentations of invited speakers from these conferences were published in special issues of distinguished journals: Regional Immunology, American Journal of Reproductive Immunology and Periodicum Biologorum. During the Meeting which was held in 1994 the Alps Adria Society for Immunology of Reproduction (AASIR) was founded as an affiliated society to the International Society for Immunology of Reproduction (ISIR). D. Rukavina was elected as a first president (2004–2007) and in the next ten years AASIR was the most active European group in reproductive immunology.

The outstanding reputation of AASIR meetings in the International Society for Immunology of Reproduction (ISIR) resulted with the organization of 8th and 10th International Congresses of Reproductive Immunology, which were held in Opatija in 2001 and 2007, respectively. In the period 2004–2007, D. Rukavina acted as the president of the ISIR.

NATIONAL AND INTERNATIONAL RECOGNITION OF IMMUNOLOGISTS' FROM RIJEKA

Scientific and public activities of Rijeka's immunologists was recognised and awarded nationally and internationally. D. Rukavina became in 2000 full member of the Croatian Academy of Sciences and Arts in the Department of Medical Sciences and was awarded in 2003 by the Republic of Croatia for lifetime achievements. Croatian annual National Prize for Science »Ruđer Bošković« was awarded to Š. Vlahović (1972), D. Rukavina (1985), S. Jonjić (1993), P. Lučin (1998), B. Polić (2002) and A. Krmpotić (2002), and for Young Researchers to G. Laškarin (2000). The Annual Award of the Croatian Academy of Sciences and Arts for Scientific Achievements was given to P. Lučin (1996), D. Rukavina (1998) and S. Jonjić (2003). The Annual Award of the City of Rijeka was given to the Department of Physiology and Immunology, Š. Vlahović and V. Frančišković (1971), to D. Rukavina (1987), S. Jonjić (1991), K. Vujaklija-Stipanović (1972 and 1994), P. Orlić (1994), and B. Radošević-Stašić (2000).

S. Jonjić was awarded in 1994 with the Günther Weitzel Science Award by the League for the development of molecular biology and biotechnology, (Tübingen, Germany) and the Raine Foundation Visiting Professorship at the University of Western Australia (2008). D. Rukavina was awarded also by the Academy of Medical Sciences of Croatia (2000), Award for scientific contribution of the government of Carinthia (1998), Award of Japanese Society for Reproductive Immunology (2000),

American Society for Reproductive Immunology (2005) and International Society for Immunology of Reproduction (2007), in which he served as president. In 2008 D. Rukavina was awarded by Blackwell Munksgaard Award, the highest award of American Society for Reproductive Immunology for outstanding contribution to the field of reproductive immunology.

RESEARCH FOCUSES AND ACHIEVEMENTS RIJEKA'S IMMUNOLOGISTS

Clinical immunology and transplantation

The group led by D. Rukavina is developing research in the field of clinical immunology and transplantation for more than three decades. This research was supported by a number of national and international research grants (UK grant ALIS in collaboration with P.M. Johnson and NIH grant in collaboration with E.R. Podack). In the period 1986–1990, D. Rukavina was coordinator of the national programme Transplantation and Clinical Immunology. Through these programs, he developed a fruitful cooperation with a number of clinics and more than 60 young researchers achieved either Master of Science of Doctoral degrees under his supervision. More than 30 are today teachers at clinics or departments of the Medical Faculty of the University of Rijeka or abroad.

Basic and clinical research programs in the field of neuroimmunology were developed in collaboration with J. Sepčić, Chairman of the Neurology Clinics. Multiple sclerosis (MS) as a clinical entity and chronic relapsing form of experimental allergic encephalomyelitis (CR-EAE) were used by M. Morović, H. Haller, D. Ledić and L. Zaputović as research models. CR-EAE as an experimental model for MS was induced in rats *(5, 6)*. The distribution of tissue compatibility antigens (HLA) was investigated in MS patients from cluster Gorski Kotar, known as an area of high prevalence for disease *(7)*.

The investigation of lymphocyte subpopulations dynamics (active T lymphocyte) and cytolytic mechanisms and cytolytic molecules expression (perforin) in the peripheral blood and cerebrospinal fluid confirmed the potential role of these mechanisms in the clinical course of disease, particularly in the phase of exacerbation. It has been suggested that $CD4^+P^+$ cytotoxic cells may play a role in the pathogenetic mechanisms of MS. These cells are upregulated in active disease in cell number, in the level of P expression per cell and in the level of cell activation *(8, 9)*.

Collaboration of the Department of Physiology and Immunology with Transplantation center at Clinical Hospital in Rijeka, which was established before the first successful kidney transplantation (1968) is lasting for four decades now *(10)*. Successful collaboration in research and clinical programs was established with clinicians from the transplantation team (P. Orlić) and Department of Transfusion and Tissue typing (K. Vujaklija-Stipanović and S. Balen-Marunić). Investigation and collaboration was centered to programs of donor selection, prediction of immunological rejection crisis, detection of immunological competence of transplanted patients, the level of immunosuppresion, effects of donor specific transfusion pre-treatment etc. It has been shown also that the mechanisms of lymphocyte cytotoxicity mediated by cytolytic molecule perforin are potentially important in allogeneic kidney rejection *(11, 12)*.

The research interest of Gordan Gulan and Jagoda Ravlić-Gulan in the field of human rheumatoid arthritis is based on intensive collaborative work among Clinic of Orthophaedic Surgery of Lovran, Clinical Hospital Centre of Rijeka and Department of Physiology and Immunology. They investigated the role of the cytolytic action mediated by perforin in the course of rheumatoid arthritis (RA) at systemic (peripheral blood) and local level (synovial fluid and synovial membrane) in patients during the acute or chronic phase of RA. In acute RA highly significant changes in P expression were found in all compartments with strong increase of P^+ cells, $CD8^+P^+$ and $CD56^+P^+$ cells as well as the content of P/cell. Strong evidence was obtained that P mediated cytotoxicity can participate in the acute phase of RA by maintaining and perpetuating the inflammation and contributing to tissue destruction *(13, 14)*.

Very interesting results were obtained by D. Rukavina and his coworkers in investigations of the role(s) of lymphocyte cytotoxicity mediated by perforin in various physiological conditions. Proportions of perforin (P) positive lymphocytes showed age-related changes with strong decline after the age of 70 years for T cells and $CD16^+$ and $CD56^+$NK cells as well as a highly significant reduction in mean levels of P per cell. All these changes resulted in the deficiency of cytotoxic potential in old age which may have implications for antiviral and antitumour immunity in elderly persons *(15)*.

In investigations of chronic hepatitis C virus infection, in collaboration with immunologists and clinicians from Pecs University (Hungary), a decreased percentage of $CD3^-CD8^+$, $V\gamma9/V\delta2TCR^+$ and perforin-posititve T cells was found with decreased peripheral NK activity which may contribute to the impaired cellular immune response and the chronicity of the disease *(16)*.

Larisa Prpić as a PhD student investigated the role played by cell–mediates cytotoxicity in the course of psoriasis and *lichen planus* at both systemic and local level (affected skin lesions). Significant accumulation of T cells was found in both epidermis and dermis of *lichen planus* lesions suggesting a potential role of perforin in the apoptosis of basal keratinocytes. Strong infiltration of T lymphocytes and accumulation of perforin was found in the epidermis of psoriatic lesions, suggesting the potential role for perforin in the creation of the psoriatic plaque and in the disease severity *(17, 18)*.

Immunology of reproduction

Immunology of reproduction and immunological relations between mother and foetus, defined by the Nobel Prize winner Peter Medawar (1953) and Rupert Billingham (1964), as a most intriguing puzzle of the transplantation immunology attracted very early research interest of D. Rukavina and later many of his coworkers. In master thesis (1968) and in doctoral thesis (1972) D. Rukavina investigated immunological aspects of the maternal-foetal relationship and found the interesting effect of maternal transplantation immunity on the specific reactivity of the offspring to the same transplantation stimuli to which the mother had been exposed. This approach was the focus of his research interest in Dr. Billingham's laboratory (Dallas, 1972/73) where he worked as a Fulbright fellow. Upon his return to Rijeka, D. Rukavina organized his own research group (M. Dorić, S. Jonjić, M. Kapović) at the Department and established close collaboration with clinicians from Department of Obstetrics and Gynaecology (N. Matejčić and J. Stašić). The research was centered to the investigation of the consequences of maternal systemic and intrauterine allogeneic sensitization to the outcome of pregnancy and the offspring reactivity to these specific stimuli. The animal models were mice and rats with haemochorial placenta *(19, 20)* and sheep with syndesnochorial placenta, which is thought as an impermeable barrier *(21)*.

Interesting results were obtained showing that pregnancies of sheep sensitized to parental partner transplantation antigens during pregnancy rejected paternal grafts as second set grafts.

In the next period the research interest was focused on the mechanisms that control embryonic implantation. Many PhD students and young clinicians were involved in the investigation of bone-marrow derived immunocompetent cells infiltrating cyclic endometrium and decidua of human early pregnancy. H. Haller and O. Petrović showed accumulation of $CD56^+$ cells in the first trimester decidua, five fold increase of CD56:CD3 cell ratio and analyzed the consequences of decidua-trophoblast interactions on the phenotype, spontaneous and induced proliferation and immunoregulatory potential of decidual leucocytes in normal and pathological pregnancies *(22, 23)*.

This group was the first showing that decidual NK cells, the predominant population of decidua infiltrating lymphocytes, are $CD56^+CD16^-$ and full of perforin (perforin $bright^+$). The cytolytic molecule perforin is expressed in first trimester pregnancy decidua in quantities higher than in any other pathological condition (tumours, inflammation) *(24)*. The investigations of the phenotype and distribution of decidua infiltrating leucocytes, expression of cytokines and cytolytic molecules, functional activity, secreted molecules such as cytokines, gene expression and protein profiles were the themes of Master and PhD theses of G. Rubeša, L. Gudelj, G. Gulan, G. Laškarin, I. Bedenicki, N. Štrbo, V. Sotošek, K. Ćupurdija, T. Bogović Crnčić and K. Juretić Franković *(25–29)*.

G. Laškarin is now leading her own research project on immunoregulatory functions of antigen presenting cells in early pregnancy. Interesting results were obtained in investigation of the role of mannose receptor (MR) in the initiation of the immune response and regulation of homeostasis during inflammation and tissue remodelling at the maternal-foetal interface. Decidual MR^+ macrophages, surrounding early decidual glands are able to internalize ligands for carbohydrate recognition domain of the receptor, including decidual secretory phase mucin TAG-72. TAG-72 efficiently downregulate Th_{12} oriented cytokine/chemokine production, whereas MUC I up-regulated pro-inflammatory decoy receptor expression *(29)*.

N. Štrbo and V. Sotošek in collaboration with E. R. Podack (Miami, USA) demonstrated an essential role for perforin-mediated functions in the activation of innate and adaptive immunity by heat shock protein gp96- peptide complexes. Cooperation between NK and dendritic cells was necessary for both NK activation and clonal CTL expansion *(30, 31)*.

In the frame of EMBIC network we examined the relative contribution to the cytotoxic function of different NK activating receptors. Specific engagement of NKp46 induced intracellular calcium mobilization, perforin polarization, granule exocytosis, and target cell lysis. This was dramatically blocked by NKG2A co-engagement. From the other side the engagement of NKp30 triggered the production of proinflammatory molecules (IFN-gamma, TNFalpha etc.) *(32)*.

In collaboration with A. Mantovani (Milano) as a part of EMBIC project, G. Laškarin and J. Dupor, PhD student participated in the investigation of decoy receptors, an emerging family of negative regulators of different classes of immune mediators. D6 molecule is the best defined chemokine decoy receptor with scavenger activity and is strongly expressed on the syncytiotrophoblast cells. D6 is strategically located as a protective barrier at maternal-fetal interface to tune inflammation by means of chemokine scavenging. Exposure of $D6^{-/-}$ pregnant mice to LPS or antiphospholipid autoantibodies results in increase in foetal loss which is prevented by blocking inflammatory chemokines *(33, 34)*.

Immune control of organ growth

Among the first projects financially supported by Republic funds (1965–1975) were the investigations of radiation biology, transplantation immunology and compensatory renal growth, led by Š. Vlahović. Subsequently a coordinator of the projects investigating the immune aspects of organ growth and neuro-endocrine influences on lymphatic tissue became B. Radošević-Stašić, with the co-workers M. Ćuk, M. Petković, L. Polić, D. Muhvić, Z. Trobonjača, J. Ravlić-Gulan and I. Mrakovčić-Šutić.

She established a very close collaboration with the researchers on Department of Chemistry and Biochemistry led by Mladena Kirigin and than by Čedomila Milin, enlarging the investigations to metabolic aspect of organ growth and immune reaction. Their early data have shown that disturbance of morphostasis results in activation of lymphatic cells with morphogenetic properties *(35, 36)*. Later, they found that the main regulators are autoreactive NKT and regulatory T cells (*37, 38*), which might be found particularly in the liver, and that these events were significantly affected by metals, particularly zinc *(39)*. Working on immunoregulatory effects of peptidoglycan-monomer linked with zinc (PGM-.Zn) and on projects supported by Pliva, they also found that PGM-Zn has immunocorrective and hepatocorrective properties in conditions of immunosuppression *(40, 41)*. These data were in 1992 protected by Pliva by the patent P920488A.

Previous members of this group are now leading their own projects, working on tumor immunology (M. Petković and I. Mrakovčić Šutić), autoimmune diseases (J. Ravlić-Gulan) and cytomegalovirus infection (Z. Trobonjača).

The new research fellows from the group of B. Radošević-Stašić, H. Jakovac and D. Grebić, subsequently showed by immunocytochemistry and PCR that activation of lymphatic cells during disturbance of morphostasis depends on damage-associated signals, and particularly on metallothioneins and endoplasmic reticulum resident heat shock protein gp96, which might be found in regenerating tissue, liver, thymus, at fetoplacental unit and in CNS during experimental allergic encephalomyelitis (EAE) *(42, 43)*. Moreover, in a current scientific project with Vladimir Mićović from Teaching Institute of Public Health, Primorsko-goranska County they demonstrated that expression of metallothioneins and heat shock proteins in the marine shells are in high correlation with environmental pollution in Kvarnerian bay.

Neuroimmunomodulation

Very early the group of B. Radošević-Stašić in coordination with Suad Efendić (Stockholm) started to investigate also the effects of somatostatin (ST) on compensatory renal growth and on the lymphatic tissue. Among the first in the world (in 1983) they reported about the immunosuppressive and growth regulatory properties of ST and its ability to modulate the graft-versus-host reaction, EAE and the processes of differentiation and proliferation in the thymus *(44–48)*.

Besides in coordination with Walter Pierpaoli and George Maestroni the group of B. Radošević-Stašić showed that pineal gland and melatonin are directly involved in the control of organ growth and immune functions, pointing to its importance for chronobiology. The group of B. Radošević-Stašić also contributed to the elucidation of the effects of cholinergic influences on immune response, showing that lesions of nucleus basalis and damage of projections to neocortex markedly affect the immune response *(49, 50)*.

Immunosurveillance of cytomegalovirus infection

As a continuation of the work that S. Jonjić performed while he was with Ulrich Koszinowski in Germany, later on, at the Department of Physiology and Immunology, he and his group were involved in studies of immunosurveillance of cytomegalovirus (CMV) infection *(51)*. Interestingly, although it has been clearly shown that $CD8^+$ T cells play a dominant role in control of CMV infection, they provided new evidence that this does not apply to all tissues. Namely, in absence of $CD4^+$ T cells, CMV infected mice develop persistent infection in salivary glands, showing for the first time the importance of $CD4^+$ T cells for the prevention of horizontal virus spread *(51)*. In subsequent series of studies it was shown that $CD4^+$ T cells mediate their effect via IFN-gamma, which is necessary for preventing the horizontal transmission of MCMV infection *(52)*. Furthermore, although $CD8^+$ T cells are the major protective subset in the control of CMV infection, mice lacking $CD8^+$ T cells eliminated virus via the compensatory response mediated by $CD4^+$ T cells *(53)*. To our knowledge this was the first example of physiological compensation in immune response. This finding was additionally confirmed by experiments in mice lacking MHC class I complexes *(54)*. It was shown that these mice can control primary CMV infection almost with the same kinetics as normal mice. Series of studies by P. Lučin, I. Pavić, B. Polić and others provided evidence that cytokines such as IFN-gamma but also TNF-alpha are important in virus control *(55, 56)*.

The group of S. Jonjić also contributed to the understanding of immunosurveillance of chronic and latent CMV infection and conditions that lead to viral reactivation from latency *(57)*. By use of B-cell deficient mice, a model to study the immunosurveillance of CMV infection in the absence of antibodies was established *(58)*. They were the first to show that antiviral antibodies are not required for the resolution of primary infection and the establishment and maintenance of CMV latency. B cell deficient mice were able to control infection and establish latency with similar kinetics as normal mice. However, the results confirmed that antiviral antibodies play a key role in limiting recurrent infection *(58)*. The results clearly demonstrated that immunosurveillance of latent CMV infection is organized in a hierarchical and redundant fashion. Namely, not only $CD8^+$ T cells but also $CD4^+$ T cells and NK cells contribute to control of MCMV latency and prevention of recurrent infection *(59)*.

An important aspect of the research was the characterization of the role of viral immunoevasins of $CD8^+$ T cells by down modulation of MHC class I molecules *(60)*. Together with Koszinowski's laboratory they provided evidence that MCMV in-

hibitors of MHC class I molecule play a role in virus control by $CD8^+$ T cells *in vivo (61)*. The deletion of the MCMV m152 gene results in virus attenuation with an impaired virulence and replication *in vivo* due to a more stringent immune control by $CD8^+$ T lymphocytes.

Viral regulation of NK cells

Guided by the observation that during the first days post infection most of laboratory mouse strains develop no significant NK-cell dependent virus control immunologists at the Department of Histology and Embryology postulated that this could be the consequence of viral immunoevasion of NK cells. As a result, over the past several years they have characterized several MCMV genes encoding proteins involved in the evasion of NK cells. Namely, it was shown that MCMV prevents NK cell activation by down-modulating cellular ligands for the activating NK cell receptor NKG2D. This group was first to describe MCMV protein involved in this function *(62)*. Apart from the downregulation of MHC class I molecules, m152 prevents expression of NKG2D ligand RAE-1 from the cell surface. Importantly, the mutant virus lacking m152 was attenuated on day 3 post-infection and the attenuation could be abolished by the depletion of NK cells *(62)*. Of note is that in the same issue of the Nature Immunology Klas Karre wrote News & Views article emphasizing the significance of this finding. In subsequent years in this laboratory three additional MCMV genes involved in downmodulation of NKG2D ligands have been characterized *(63–65)*. Whereas m152 is involved in down-regulation of RAE-1 isoforms, the m155 down-modulates H60 and m145 negatively regulates the expression of MULT-1. Interestingly, the product of *m138* gene, originally characterized as viral receptor for Fc fragment of immunoglobulins, is involved in down-regulation of at least two NKG2D ligands – H60 and MULT-1. It was previously shown that the virus lacking *m138* is strongly attenuated during the early days post infection even in immunoglobulin-deficient animals suggesting that Fc binding is not the only immunoevasion property of this protein (66). In addition to molecular and functional characterization of viral NKG2D immunoevasins it was shown that they play a role *in vivo (67, 68)*.

Currently, in frame of HHMI research fellowship, A. Krmpotić and colleagues are studying the significance of viral immunoevasins of innate immunity for the long-term control of CMV infection.

Apart from work on the characterization of viral inhibitors of NKG2D, the Jonjić's group took part in the characterization of MCMV proteins involved in activation of NK cells. Others have shown that MCMV m157 protein serves as a ligand for activating Ly49H receptor C57BL/6 mice. By deleting m157 gene, the group from Rijeka together with Koszinowski and colleagues have shown that the entire NK cell control of the virus was abolished, suggesting that no other MCMV protein is able to engage Ly49H *(69)*. Of note is that under selective pressure by Ly49H the m157 gene is subject for mutation leading to mutant strains able to escape NK cell control *(70)*. Apart from Ly49H, in a very recent study, this group took part in the collaborative research with Silvia Vidal aimed at characterization of another viral protein involved in NK cell activation via Ly49P receptor (*J Exp Med* submitted). It was shown that MCMV m04 is essential for the recognition of infected cells by Ly49P in MA/My mice. Consequently, the deletion of m04 resulted in loss of virus susceptibility to NK cells. Interestingly, unlike Ly49H, for the activation of Ly49P in addition to viral protein the specific MHC class I molecule is required. The results in PWK mouse strain also indicate that NK cell dependent resistance of this strain to MCMV also includes recognition of so far unknown viral protein (*71*).

Congenital CMV infection of developing CNS

More recently, the mouse CMV as a model to study human CMV infection in the developing CNS was established through collaboration between Jonjić's group and Bill Britt at the University of Alabama, Birmingham and supported by NIH RO1 Grant *(72)*. Congenital HCMV infection of CNS represents one of the major viral causes of congenital abnormalities in CNS. Inoculation of newborn mice with MCMV resulted in virus spread to the brain and the infection was associated with the induction of inflammatory response (focal encephalitis) and defects in cerebellar development. Specific defects included decreased granular neuron proliferation and migration and the activation of neurotrophil receptors. Ongoing studies in this laboratory and collaborators have already proven that this model is going to play an important role in understanding the immunobiology of congenital CMV infection *(72)*.

The role of NKG2D and other immunoreceptors on the development, homeostasis and effector functions of the immune system

B. Polić, upon his return from the postdoctoral education in Germany, has established mouse gene targeting program at the Department of Histology and Embryology (2002), which was a pioneering endeavour in Croatia. This program has been giving to scientists a possibility to genetically create new mouse models to answer fundamental biological questions, and thus,

an additional flexibility and quality to the present research at the Medical Faculty. He and his co-workers have produced, among the others, the conventional and conditional mouse mutants for gene encoding NKG2D receptor, which are now under the scope of investigation. NKG2D, as an activating receptor, is present on various immune cell populations (NK, NKT, and T) and, according to the present literature; it is implicated in their effector functions. The analysis of NKG2D knock out mice has revealed impaired NK cell development as well as homeostasis and effector functions of NK cells, which is going to be reported soon. Interest of this group is to further investigate the role and mechanisms of action of this and other immunoreceptors in innate and adaptive immunity (Figure 5).

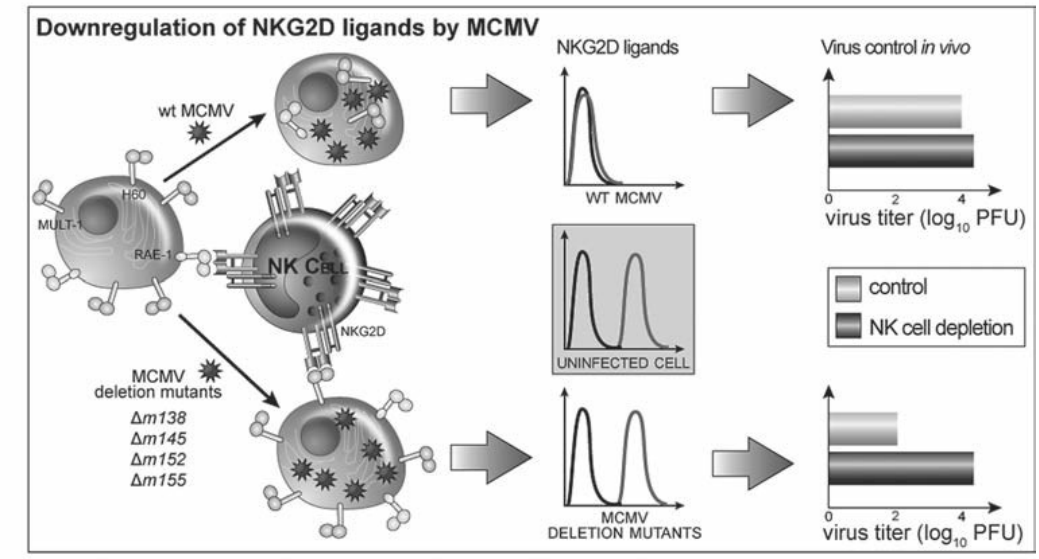

Figure 5. ***MCMV inhibitors of NKG2D ligands*** *(Jonjić et al., Current Opinion in Immunology, 2008).*

Cell biology of murine cytomegalovirus infection

Cell biology of murine cytomegalovirus infection was for a decade in the background of immunological research. In the 1994, P. Lučin described synergistic inhibitory effect of interferon-gamma and tumour necrosis factor on the late phase of murine cytomegalovirus replication *(56)*. Their effect was also extended on restoration of defective antigen presentation *(55)*. Natalia Kučić demonstrated that MCMV replication requires activity of protein kinases C in the early phase *(73)*.

Murine cytomegalovirus interference with MHC class I molecules

Immune evasion mechanism of murine cytomegalovirus has been studied from several aspects. The initial observations were made within research project of S. Jonjić in collaboration with Ulrich H. Koszinowski on presentation of MCMV antigens to cytotoxic lymphocytes. In the 1992, it was published that cytomegalovirus prevents antigen presentation by blocking the transport of peptide-loaded major histocompatibility complex class I molecules into the medial-Golgi compartment *(74)*. Pero Lučin continued research in Ulm and Heidelberg (1992–1993) and upon return in Rijeka established independent group that was joined by N. Kučić in 1995, and Hana Mahmutefendić in 1999. First attempt to identify the MCMV gene responsible for the antigen presentation block vas unsuccessful, but led to the discovery of a MCMV gene with Fc receptor function *(75)*. The MCMV genomic region affecting MHC class I molecule transport *(76)* was first identified and then MCMV glycoprotein m152 that retains MHC class I complexes in the ERGIC/cis-Golgi compartments *(77)*. In addition to this protein, two MCMV proteins that affect MHC-I transport were identified: gp48, that reroutes MHC class I complexes to lysosomes for degradation *(78)*, and gp34 that forms a complex with folded class I MHC molecules in the ER which is not retained but transported to the cell surface *(79, 80, 81)*.

Initial observation that MCMV also affects MHC-I molecules at the cell surface and cause their backsorting was published in 1994 *(81)*, but attempts to characterize mechanism were unsuccessful. At that time, the overall expertise in the endocytic system were poor in Rijeka and Lučin's group shifted research focus towards endocytic trafficking of MHC-I molecules.

Endocytic trafficking of MHC class I molecules

Studies of endosomal trafficking of MHC class I molecules were initiated in 2000 when H. Mahmutefendić joined the group of P. Lučin. Early research indicated that MHC-I sorting depends on their conformation *(82),* and spontaneous internalization as a consequence of the bulk cellular membrane flow demonstrated that conformed and nonconformed MHC-I molecules use different internalization and trafficking route *(83)*. In 2003, the group was joined by Gordana Blagojević, who established model of cholera toxin endocytic trafficking *(84),* and studying trafficking of human MHC-I molecules in transfected cells. In the 2005, Maja Ilić Tomaš joined the group and continued research of endosomal sorting of MHC-I molecules in MCMV infected cells.

Immunopathogenesis of bacterial infections

The group led by M. Dorić has a long standing interest in scientific research on the pathogenesis of bacterial infections. In the period 1988–1991, an original computer-assisted data base system for identification of gram negative nonfermentative and fermentative bacteria was constructed, followed by development of 11 computer-based programs for bacterial and yeasts identification in the period 1991–1996. At that time antibacterial effectiveness was correlated with the effect of antibiotics on the host defence system (lymphocyte subpopulations, antibody secretion, macrophages and neutrophils) which resulted with four Master Thesis mentored by M. Dorić and two original scientific papers *(85, 86)*. In the 1997, as a continuation in the field of bacterial pathogenesis and immunology of reproduction, M. Dorić started research on pathogenesis of experimental murine congenital listeriosis. Murine model of pregnancy-associated listeriosis and the host immune response to *L. monocytogenes* infection was established by M. Abram *(87),* and *in vivo* model of *Campylobacter jejuni* infection by, D. Vučković. Proinflammatory cytokine response to these two pathogens indicated that IFN-γ and TNF-α production correlate with bacterial clearance from the liver *(88)* and that, in contrast to *L. monocytogenes*, *C. jejuni* does not promote induction of IL-6 *(89)*.

T. Rukavina produced monoclonal antibodies against *Klebsiella* capsular polysaccharides *(90)* and showed their neutralizing and protective effect *(91, 92)* in the murine model of lung and systemic *Klebsiella* infection *(93)*. B. Tićac showed that the ability of *Legionella pneumophila* to cause pneumonia depended on its capacity to invade and replicate within alveolar macrophages, monocytes, and potentially alveolar epithelial cells *(94)*. Vanja Vasiljev-Marchesi joined T. Rukavina group in 2002 and demonstrated that reduced cytokine production was involved in the survival of animals protected by antilipopolysaccharide antibodies *(95)*. The ratios between IL-10 and proinflammatory cytokines confirmed the suppressed pro-inflammatory response in protected animals, especially 24 hours postinfection *(96)*.

Marina Šantić, a member of the M. Dorić group, continued with *Legionella* research and showed that the rsmA, htrA and ligA genes are essential for the replication of *L. pneumophila* and for the expression of mRNA for IL-1α and IL-18 *(97)*. Ivana Gobin, who came in 2001, compared the pathogenic potential of *L. pneumophila* and *L. longbeache*, showing that there are significant differences in the infective doses, permissiveness of experimental animals and pathohistological changes in the lung tissue This group also showed that Dot/Icm type IV secretion system is essential not only for modulation of phagosome biogenesis but also for the activation of caspase-3 *(98, 99)*. When comparing intracellular survival of different bacterial species, the research was enlarged to pathogenesis of *Francisella tularensis subsp. novicida (100, 101)*.

The M. Abram's group focused on cellular and molecular mechanisms in the pathogenesis of congenital listeriosis and demonstrated that listeria could traverse the placenta and cause serious foetal damage by immunopathological mechanisms *(102, 103)*. M. Bubonja, a member of the group, established a mouse model that mimic the natural route of *L. monocytogenes* infection, showing that intragastric administration of listeria for three consecutive days led to the development of severe systemic illness with involvement of brain tissue in all infected mice. Comparison with campylobacteriosis revealed that *C. jejuni* is not typical extracellular bacterium *(104)*.

Experimental autoimmune models

The main animal model for investigation of pathogenesis of multiple sclerosis was experimental allergic encephalomyelitis (EAE), introduced at the Department of Physiology and Immunology by D. Rukavina and coworkers in this field – V. Barac-Latas, M. Morović and D. Muhvić. It was induced in its relapsing and monophasic form in AO (resistant) and DA (susceptible) strain of rats, forming a basis for investigations of mechanisms that induce the chronic inflammatory demyelinating disease. Working in the group of H. Lassmann in Vienna, Vesna Barac-Latas studied also the patterns of oligodendrocyte pathology in coronavirus-induced subacute demyelinating encephalomyelitis in the Lewis rat, showing that infected oligodendrocytes were destroyed by necrosis, whereas oligodendrocytes that did not contain detectable virus antigen or RNA were dying by apoptosis *(105)*. D. Muhvić later showed that some of early manifestations might be changed by peripheral or intracerebroventricular application of somatostatin *(46, 47)*.

Using the streptozotocin-induced autoimmune diabetes as a model for diabetes mellitus type I, I. Mrakovčić-Šutić showed that it involves high accumulation of NKT cells in the liver with increased cytotoxicity against syngeneic thymocytes *(38)*.

Studies on ethiopathogenesis of the inflammatory bowel disease were introduced by Z. Trobonjača when he moved to Ulm, Germany and joined group of Joerg Reimann. They published interesting observation that small population of spleen $CD4^+$ class I-restrictive cells can induce extremely aggressive and lethal colitis in immunodefficient RAG KO host mice *(106)*. Simultaneously to colitis investigation this group carried out experiments on liver antiviral immunity that were partially completed in Rijeka. They showed importance of dendritic cells in the activation of hepatic NKT cells *(107, 108)* and a mechanism of crosstalk between liver NK, NKT and DCs that is multiplying hepatic IFN-gamma production *(109, 110)*.

CONCLUDING REMARKS

Four decades of investment in people and their training at the Department of Physiology and Immunology created the group of immunologists in Rijeka that have international scientific reputation and significant impact on development of biomedicine in Croatia. As an open training centre with creative and productive spirit, Department of Physiology and Immunology substantially contributed to the development of the Medical Faculty of Rijeka. Dissemination of early doctorants to the chairs of a number of departments and clinics in Rijeka, as well as collaboration with numerous scientists outside department helped in profiling the scientific mindset in the Rijeka's academic community. Group at the Department of Histology is nowdays one of the most productive groups in Croatia, but similar pattern of scientific behaviour is growing also at microbiology, biology, biochemistry and many other departments. This paper, made on the occasion of the 40 years of Croatian Immunological Society, is and opportunity to thank to the founders of immunology in Rijeka: to Šime Vlahović, Vinko Frančišković and Daniel Rukavina. Their vision, creative spirit and entrepreneurial energy made an adamant base for the competitive research environment of the University of Rijeka.

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CHRONOLOGY OF IMPORTANT DATES

1972 Foundation of the Tissue Typing Centre headed by Andrija Kaštelan (1970–2001) and Vesna Brkljačić-Kerhin (2001–now).

From 1970–2007 the center was part of the Urology Clinic of the Clinical Hospital Center Zagreb and Zagreb University School of Medicine.

In 2007 the center became part of the Clinical Institute of Laboratory Diagnosis and changed its original name to Department for Tissue Typing.

1977 Foundation of the Laboratory for Cellular Immunology headed by Maja Kaštelan (1977–1981). From 1977–1981 the laboratory was part of the Oncology and Radiotherapy Clinic, Clinical Hospital Center Zagreb.

From 1981–1985 the laboratory was part of the Division of Immunology within the Department for Clinical Laboratory Diagnostic of the Clinical Hospital Center Zagreb.

1978 Foundation of the Division of Clinical Immunology and Inflammatory Rheumatic Diseases as a part of the Clinic for Internal Medicine, Clinical Hospital Center Zagreb and Zagreb University School of Medicine; the division was headed by Zvonimir Horvat (1978–1990).

Foundation of the Laboratory for Serologic Immunodiagnostics as a part of the Division of Clinical Immunology and Inflammatory Rheumatic Diseases, also headed by Zvonimir Horvat (1978–1981).

1981 Foundation of the Department for Clinical Laboratory Diagnostic (so called »central laboratory«) of the Clinical Hospital Center Zagreb headed by Ana Stavljenić Rukavina (1981–1985).

Laboratory for Cellular Immunology and Laboratory for Serologic Immunodiagnostics were united and became a new organizational unit – Division of Immunology – within the Department for Clinical Laboratory Diagnostics, Clinical Hospital Center Zagreb. The head of this new division was Maja Kaštelan.

1985 Department for Clinical Laboratory Diagnostics of the Clinical Hospital Center changed its name to the Department for Clinical Laboratory Diagnostics of the Zagreb University School of Medicine (headed by Ana Stavljenić Rukavina (1985.–1992.)

1986. Matko Marušić, a professor of physiology and immunology from the Zagreb University School of Medicine became head of the Division of Immunology of the Department for Clinical Laboratory Diagnostics.