



Materials on successful workshop accomplishment, including guidelines

Deliverable D2.1

VirA project No. 952376

New trends in autoimmune diseases immunological profiling

Projec	Project funded by the European Union		
Disser	Dissemination Level		
PU	Public	Х	
PP	Restricted to other program participants (including the Commission Services)		
RE	Restricted to a group specified by the consortium (including the Commission Services)		
СО	Confidential, only for members of the consortium (including the Commission Services)		

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Introduction and overview of the workshop

The three-day VirA project workshop "New trends in autoimmune diseases immunological profiling" intended to foster theoretical and practical knowledge on autoimmune disease research and international knowledge transfer among experts with the aim to bolster long-term interdisciplinary collaboration.

According to the project schedule, the workshop had to be carried out at a time when it was challenging form an epidemiological standpoint; i.e. by following COVID-19 restrictions in each participant country. Therefore, a decision was made to split the workshop in two parts - theoretical (October 27-28, 2021) and practical (July 16-17, 2022).

Part one was held remotely with more than 40 participants dialling-in, including undergraduates, PhD students and young researchers, as well as clinicians and specialists working on autoimmune pathologies.

Theoretical lectures included a broad range of topics, from mechanisms leading to autoimmune diseases to single cell sub-typing in autoimmune diseases and DNA sensing and autoimmunity. Specific laboratory methods, which are applied in autoimmune disease research using cell cultures and peripheral blood, were demonstrated through video feeds. The lecturers included experienced scientists from the VirA participating countries Italy, Israel and Germany, as well as a guest lecturer from the Netherlands. The experts gave overviews of current challenges in autoimmune disease diagnostics, immunocompetent cell phenotyping and shared the experience from their institutes in tackling these issues. The young researchers also had the opportunity to hold short presentations of their own work and discuss topical questions with their senior colleagues and fellow experts from the aforementioned partner countries.

Part two of the workshop was aiming to benefit from an improved epidemiological situation in Europe, allowing for easier travel and in-person meetings to really maximise on the efficiency of interactions and focus on practical skills, discussions with experts during work process and simply allow for more engaging face-to-face conversations.

Theoretical portion of this part of the workshop included topics around COVID-19 infections in relation to effects of autoimmune diseases, however the emphasis was on flow cytometric analysis of autoimmune phenotypes, autoantibody profiles against interferons and cytokines in patients with autoimmune diseases and laboratory diagnostics of autoantibodies. Practical work was intended to be held in five to six people working groups in the laboratory, however, unfortunately, one of the lecturers showed signs of acute respiratory tract infection on the first day and, after testing, was diagnosed with COIVD-19.

The remainder of the workshop was rescheduled according to the then-current epidemiological restrictions and partially continued online and partially in person with use of protective gear and social distancing, including working from separate rooms that were outfitted with equipment for online conferencing. However, as the workshop was adopted to the newly developed situation, the planned laboratory work could not be held.

Our colleagues form Germany, Israel and Italy delivered the theoretical lectures in full, over remote and in-person formats, and actively discussed protocols of fluorescence-activated cell sorting and analyses, giving suggestions for improvements and diversification. Auto-antibody and proinflammatory cytokines expression profile detection based on biomarker analysis platforms ELISA and Luminex was likewise widely discussed.

In summary, there were 40 participants in part two of the workshop "New trends in autoimmune diseases immunological profiling", including researchers, undergraduate students and PhD students, the young researchers, as well as clinicians and laboratory specialists. The workshop program was carried out by abiding to all epidemiological safety requirements.

The agenda of the workshop and a list of attendees can be found in Annex 1 and Annex 2, respectively.

Overview of the key presentations

Part I, October 27-28, 2021

Why autoimmunity



Yehuda Shoenfeld (Professor, Tel-Aviv University, President of Ariel University, Israel) accentuates autoimmunity as a problem that is becoming ever more important and affects more and more people. Two types of autoimmune reactions were highlighted: firstly – those that pertain to autoimmune reactions caused by adjuvants - a substance that enhances the immune system's response to the presence of an antigen. It could lead to the development of the

autoimmune/inflammatory syndrome induced by adjuvants (ASIA), also known as Shoenfeld's syndrome and in genetically susceptible individuals, adjuvant administration can lead to overt autoimmune disease induction, as well as autoantibody production, and secondly – autoantibodies formation to autonomic nervous system receptors.

The effect of contemporary therapeutic immunomodulating agents on the development of autoimmune processes was also analyzed, especially in the context of immune check-point inhibitors. The need for predictive and prognostic biomarkers of the autoimmune diseases was discussed.



Mechanisms leading to autoimmune diseases

Miri Blank (Professor Department of Microbiology and Clinical Immunology, Sackler faculty of Medicine, Tel-Aviv University, Israel) described main mechanisms involved in induction of autoimmunity in genetically prone individuals: genetic predisposition, clonal escape from clonal deletion and anergy, epigenetic changes and dysregulation of T regulatory cells and cytokine network. Requirements for the development of autoimmune disease following an initial activation of auto reactive T cells were discussed. Conclusions about autoimmunity stimulating mechanisms summarized in the lecture encourages to connect genetic, epigenetic and cellular factors into a single pathogenesis chain which should then be investigated further.

Revolutionary advances for single cell subtyping in autoimmune diseases



Christophe Fleury (PhD, 10xGENOMICS, 2316XC Leiden, The Netherlands) presented new technologies allow researchers to go deeper inside the cell using a microfluidics-based method of single-cell RNA sequencing. These technologies allows for targeting of thousands of cells per sample and obtaining single-cell data with multiple dimensions. Possibilities to detect single cell

gene expression with the aim to identify different cell types, determine heterogeneity of the sample and compare sample before and after treatment were discussed. The listeners were introduced to the single cell immune profiling, which allows scientists to collect data about the immune repertoire and gene expression, reveal immune cell clonality, diversity, antigen specificity, and cellular context, characterize individual T-cells and B-cells, pair α and β chain TCR sequences from individual T-cells and pair heavy and light chain immunoglobulin sequences from individual B-cells. These advanced methods open up new possibilities in the field of autoimmune disease research.



Dendritic cells – new look at the old sentinels of the immune system

Kristīne Vaivode (MSc, Medical Microbiology, PhD, Immunology, Latvian Biomedical Research and Study centre, Riga, Latvia) at the first part of her presentation described human dendritic cells heterogeneity and at the second part new DC subsets and autoimmunity were discussed. The role of pro-inflammatory CD14+ DC3 expansion was analysed in the context of SLE and correlation with disease activity was found. CD14+ DC3 could be the subject of the future studies in autoimmune diseases.

Antigen expression in autoimmune diseases



Roberta Rizzo (Professor, Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, Italy) discussed the topic in-depth using multiple sclerosis as an example, accentuating the molecular basis and genetic aspects and s-HLA-G and s-HLA-E immunomodulatory role in MS disease activity acting as antiinflamatory molecules – suppression of effector immune cells (T and NK) and modification of citokyne environment.

DNA sensing and autoimmunity



Daria Bortolotti (PhD, Assistant Professor, Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, Italy) focused on immune system peculiarities in autoimmune neurological diseases, described NK cells functional characteristics in the presence of HHV-6 un HHV-7 infection and the role of IFN in activation of immune response leading to autoimmunity.

In this part of the workshop lectures also were given by

Gilad Halpert (PhD., Zabludowicz Center for Autoimmune Diseases, Sheba Medical Center, Israel) Autoimmune dysautonomia in women with silicone breast implants.



Miri Blank (Professor, Department of Microbiology and Clinical Immunology, Sackler faculty of Medicine, Tel-Aviv University, Israel) **Helminths derivative to treat autoimmunity.**



Marion Schneider (Prof., Dr. rer. nat., Head of the Division Experimental Anesthesiology University hospital Ulm, Department of Anaesthesiology and Intensive Care, Ulm, Germany) Interferon antibodies in patients with COVID-19 and hemophagocytic lymphohistiocytosis (HLH).



The workshop continued with a video demonstration by colleagues from University of Ferrara on how to work with cell cultures and ELISA assay.



Scientists from RSU presented their studies "Proportions of the peripheral blood mononuclear cells in patients with rheumatoid arthritis", "Selection of biomarkers in ME/CFS for patient stratification and treatment surveillance/optimization", discussed the methods used in the report "The use of the Luminex system in the context of autoimmunity research" as well as the possible paths the investigation could take in the future.

At the end of each day the results were summarized and possible further cooperation in the field of the immune processes of autoimmune pathology research were discussed.





Part II, July 16-17, 2022



Innate immune response to SarS-CoV-2 infection



Daria Bortolotti (PhD, Asst. Prof., Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, Italy) described immune response during SARS-CoV-2 infection. Besides the well-known function of lymphocytes during SARS-CoV-2 infection, also the innate immune system is an important factor in controlling the infection. In particular, Natural Killer cells represent the first line of defence against viruses, and together with RNA sensors, might be involved in typical COVID19 immune deregulations.



Flow Cytometric analysis of autoimmune phenotypes

Marion Schneider (PhD, Prof., Head of the Division Experimental Anesthesiology, Ulm University Hospital, Department of Anesthesiology and Intensive Care, Ulm, Germany). A map of metabolic phenotypes in patients with myalgic encephalomyelitis/chronic fatigue syndrome was analysed. IFN gamma expressing lymphocytes, CD4+25+ T regulatory cells, CD4+/CXCR5 T

follicular helper cells (Tfh), TH17 cells in autoimmunity were analysed by flow cytometry, results were demonstrated in graphs and differences between patients with short course of the disease and long-lasting disease and autoimmunity was shown, laboratory protocols were discussed.



Multiplex technology for biomarkers determination

Boris Gilburd (MD, PhD, Zabludowicz Center for Autoimmune Diseases, Sheba Medical Center, Tel HaShomer, Israel). The possible advantages of multiplexing immunoassays were described - the simultaneous measurement of different substances from a single sample is an emerging area for achieving efficient and high-throughput detection in several applications. Although multiplex assays have many advantages over conventional assays, there are also problems that may cause apprehension among clinicians and researchers. Multiplex technology is considered to be beneficial for the simultaneous detection of different autoantibodies related to autoimmune diseases. The autoantibody profiling of patients may be useful for determining the concentration of specific autoantibodies, which may display different trends over time, both for diagnosis and prognosis, e.g. celiac disease, anti-phospholipid syndrome (APS) etc. which are characterized by the presence of autoantibodies of different isotopes.

Work in laboratory

Practical aspects of flow cytometry assay and multiplexing immunoassays were discussed in virtual meeting. Assay protocols and nuances of interpretation were demonstrated as well.

Methods:

Flow cytometry. Immune cells phenotyping

Daria Bortolotti and **Valeria Gentili** from University of Ferrara and **Irina Kholodnuka** from RSU covered this section.

The participants were able to discuss the possibilities of the flow cytometry method for immunotyping of cells involved in autoimmune processes, paying special attention to the detection of activation markers of T ly and NK cells. Histograms of detected cells were also shown and IFN secreting cells evaluation was discussed.

Chemiluminescence based assay. Detection of autoantibodies, interpretation of the results

Boris Gilburd continued topic about possible advantages and disadvantages of multiplexing immunoassays in autoimmune diseases, the process of the test and the interpretation of obtained results were discussed.



The workshop concluded with discussions about more effective use of laboratory methods in diagnostics and surveillance of autoimmune diseases, nuances of laboratory protocols, further collaboration when learning to work with the newest technologies. The results of previously conducted laboratory research where discussed and analysed, the day's activities were summarized.





Feedback

Part I

20 participants gave the feedback.

Overall assessment of the event was 4.5 (out of 5.0).

All responders agreed that workshop achieved the programme objectives.







Part II

21 participants gave the feedback.

Overall assessment of the event was 4.57 (out of 5.0).

20 out of 21 responders agreed that workshop achieved the programme objectives.

18 out of 21 responders met their expectations from participation in the workshop.



One respondent remarked that, due to the COVID restrictions, the practical work – the essential part of a workshop – could not be performed accordingly.

Conclusions

The workshop "New trends in autoimmune diseases immunological profiling" assisted to reach the goals of the project:

- Promote existing personnel competences in theoretical and practical immunology for advanced autoimmune disease research;
- Master new approaches of autoimmunity analysis;
- Promote and strengthen collaboration inside the multidisciplinary unit;
- Establish personal contacts with researchers from partnering countries to facilitate RSU integration in ERA.

As part of the process, current topics in the field of research of autoimmune diseases were discussed, the participants of the workshop were introduced to the scientific interests of project partners, current research topics in the field of autoimmune pathologies both in the world and in partner institutions as well as the most advanced technologies and methods used. Online discussions about further research and new methods in autoimmune diseases immunological profiling were initiated. Due to COVID-19 infection it was impossible to conduct practical sessions in laboratories as planned, the faculty substituted this with theoretical lectures describing the laboratory methods, the process of analysis and the interpretation of results. In person workshop, however, would provide better engagement between the listeners and the experts as well as discuss ideas and clear up questions immediately.

Guidelines to "New trends in autoimmune diseases immunological profiling"

- Further practical laboratory work and consultations with experts on laboratory best-practices are recommended to be carried out in-person. The benefits in learning experience simply are greater and the collaboration is more productive with physical proximity.
- Feedback on the acquired knowledge during workshop should also be available in each of the home laboratories e.g. thematic discussion, small seminar, practical demonstration.
- The relatively broad range of subjects could be narrowed towards RSU's specific scientific focuses in autoimmune pathology research.
- To foster a common foundation for further research applications and common scientific publications in the field, more time could be spent on particularly those autoimmunity topics that are shared among all VirA project researchers.





EU Horizon 2020 Twinning project

Reducing networking gaps between Rīga Stradiņš University (RSU) and internationally-leading counterparts in viral infection-induced autouimmunity research

Workshop by online Zoom meeting platform

NEW TRENDS IN AUTOIMMUNE DISEASES IMMUNOLOGICAL PROFILING

Rīga Stradiņš university, Dzirciema street 16, Riga, Latvia 27 - 28th October, 2021

Workshop leader Assoc. Professor Simona Doniņa

AGENDA

October 27, 20	021		
13.00 - 13.10Welcome and opening			
	Liene Ņikitina-Zaķe, Director of Research Department, RSU, Latvia Modra Murovska, Assoc. Professor, Project Coordinator, Institute of Microbiology and Virology, RSU, Latvia		
13.10 - 14.05 Why autoimmunity.			
	Yehuda Shoenfeld, President of Ariel University, Professor, Zabludowicz Center for Autoimmune Diseases, Sheba Medical Center, Israel		
14.05 - 15.05	Mechanisms leading to autoimmune diseases.		
	Miri Blank , Professor, Department of Microbiology and Clinical Immunology, Sackler faculty of Medicine, Tel-Aviv University, Zabludowicz Center for Autoimmune Diseases, Sheba Medical Center, Israel		
15.05 - 15.20	Break		
15.20 - 16.05	Revolutionary advances for single cell subtyping in autoimmune diseases		
	Christophe Fleury, PhD, 10xGENOMICS		
16.05 16.45	Dendritic cells – new look at the old sentinels of the immune system.		
16.05 - 16.45	Kristīne Vaivode, MSc, Medical Microbiology, PhD, Immunology, Latvian Biomedical Research and Study centre, Riga, Latvia		
16.45 - 17.00	Discussion, Q&A		
	Y. Shoenfeld, M. Blank, M. Schneider, C. Fleury, R. Rizzo, K. Vaivode		





October 28, 202	21
10.00 - 10.40	Antigen expression in autoimmune diseases.
	Roberta Rizzo , Professor, Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, Italy
10.40 - 11.10	Autoimmune dysautonomia in women with silicone breast implants.
	Gilad Halpert, Dr., Zabludowicz Center for Autoimmune Diseases, Sheba Medical Center, Israel
11.10 - 11.40	Helminth derivative to treat autoimmunity.
	Miri Blank, Profesor, Department of Microbiology and Clinical Immunology, Sackler faculty of Medicine, Tel-Aviv University, Zabludowicz Center for Autoimmune Diseases, Sheba Medical Center, Israel
11.40 - 11.50	Break
11.50 - 12.30	DNA sensing and autoimmunity.
	Daria Bortolotti , PhD, Assistant Professor, Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, Italy
12.30 - 13.00	Interferon antibodies in patients with COVID-19 and hemophagocytic lymphohistiocytosis (HLH)
	Marion Schneider, Prof., Dr. rer. nat., Head of the Division Experimental Anesthesiology University hospital Ulm, Department of Anaesthesiology and Intensive Care, Ulm, Germany
13.00 - 13.50	Break
13.50 - 14.10	Cell cultures.
	Roberta Rizzo , Professor, Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, Italy
14.10 - 14.30	ELISA assay.
	Daria Bortolotti , PhD, Assistant Professor, Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, Italy
14.30 - 14.50	The use of the luminex system in the context of autoimmunity research.
	Lība Sokolovska , Research Asistant, PhD Student, Institute of Microbiology and Virology, Rīga Stradiņš university, Riga, Latvia





14.50 - 15.10	Research in immunogenetics today.
	Vladislavs Jasinskis, Researcher, Laboratory of Clinical Immunology and Immunogenetics, Institute of Microbiology and Virology, Rīga Stradiņš University, Riga, Latvia
15.10 - 15.20	Break
15.20 - 15.50	Ways to dissect inflammatory and autoimmune phenotypes in psychiatric disorders (AF and SZ).
	Christian Scheiber, M. Sc., PhD student (Mol Med.), Ulm, Germany
15.50 - 16.10	Proportions of the peripheral blood mononuclear cells in patients with rheumatoid arthritis.
	Irina Kholodnuk , PhD, Lead Researcher, Institute of Microbiology and Virology, Rīga Stradiņš university, Riga, Latvia
16.10 - 16.30	Selection of biomarkers in ME/CFS for patient stratification and treatment surveillance/optimisation. What we have done so far and further tasks.
	Anda Vilmane, Msc. biol., PhD Candidate, Researcher, Institute of Microbiology and Virology, Rīga Stradiņš university, Riga, Latvia
16.30 - 16.50	Discussion, Q&A, closing
	Y.Shoenfeld, R.Rizzo, M.Schneider, M.Blank, G.Halpert, D.Bortolotti, I.Kholodnuk, C.Scheiber, L.Sokolovska, A.Vilmane, V.Jasinskis





Workshop

New trends in autoimmune diseases immunological profiling: Part II

Rīga Stradiņš University, Institute of Microbiology and Virology 5 Rātsupītes Street, Riga **16th and 17th July, 2022**

Workshop leader PhD, Lead Researcher Simona Doniņa

AGENDA

Day/Date	Time GMT+3	Activity
Saturday, 16 July	08.30 - 09.00	Registration
	09.00 - 09.10	Opening & Welcome Modra Murovska, PhD, MD, Assoc. Prof., Project Coordinator, Institute of Microbiology and Virology, RSU, Latvia
	09.10 - 09.35	Innate immune response to SarS-CoV-2 infection Daria Bortolotti , PhD, Asst. Prof., Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, Italy
	09.35 - 10.00	HLA-G expression and CMV infection during pregnancy Valentina Gentili , PhD, Asst. Prof., Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, Italy
	10.00 - 10.25	Flow Cytometric analysis of autoimmune phenotypes Marion Schneider , PhD, Prof., Head of the Division Experimental Anesthesiology, Ulm University Hospital, Department of Anaesthesiology and Intensive Care, Ulm, Germany
	10.25 - 10.40	Coffee break

10.40 - 11.05	Autoantibody profiles against Interferons and cytokines in patients with depression, virus infections, CFS-ME and MDD Marion Schneider , PhD, Prof., Head of the		
	Division Experimental Anesthesiology, Ulm University Hospital, Department of Anaesthesiology and Intensive Care, Ulm, Germany		
11.05 - 11.30	Tolerogenic cellular therapies for autoimmune diseases and COVID-19 Miri Blank , PhD, Prof., Zabludowicz Center for Autoimmune Diseases, Sheba Medical Center, Tel HaShomer, Israel		
11.30 - 12.00	Autoantibodies and laboratory diagnosics of autoantibodies Boris Gilburd, PhD, MD, Zabludowicz Center for Autoimmune Diseases, Sheba Medical Center, Tel HaShomer, Israel		
12.00 - 13.00	Discussions, Q & A Lunch		
Practical work in	the laboratory		
13.00 16.00	Methods: Flow outometry, Immune colls		
13.00 – 16.00	Methods: Flow cytometry. Immune cells phenotyping Daria Bortolotti , PhD, Asst. Prof., Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, Italy Valentina Gentili , PhD, Asst. Prof., Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, Italy Host – Irina Holodņuka , leading researcher, Institute of Microbiology and Virology, RSU		
	autoantibodies, interpretation of the results Boris Gilburd , PhD, MD, Zabludowicz Center for Autoimmune Diseases, Sheba Medical Center, Tel HaShomer, Israel Host – Lība Sokolovska , acting researcher, scientific assistant, Institute of Microbiology and Virology, RSU Identification of pathogenic and protective epitopes,		
	using phage display libraries Miri Blank , PhD, Prof., Zabludowicz Center for Autoimmune Diseases, Sheba Medical Center, Tel HaShomer, Israel		
14.30 - 14.50	Coffee break		

Sunday,	Practical work in	the laboratory
17 July	09.00 - 12.00	Methods: Flow cytometry. Immune cells
		phenotyping
		Daria Bortolotti, PhD, Asst. Prof., Department of
		Chemical, Pharmaceutical and Agricultural
		Sciences, University of Ferrara, Italy
		Valentina Gentili, PhD, Asst. Prof., Department of
		Chemical, Pharmaceutical and Agricultural
		Sciences, University of Ferrara, Italy
		Host – Irina Holodņuka, leading researcher,
		Institute of Microbiology and Virology, RSU
		Chemiluminiscent based assay. Detection of
		autoantibodies, interpretation of the results
		Boris Gilburd, MD, PhD, Zabludowicz Center for
		Autoimmune Diseases, Sheba Medical Center,
		Tel HaShomer, Israel
		Host – Lība Sokolovska, acting researcher,
		scientific assistant, Institute of Microbiology and
		Virology, RSU
		Identification of pathogenic and protective epitopes,
		using phage display libraries
		MIRI Blank, PhD, Prof., Zabludowicz Center for
		Autoimmune Diseases, Sneba Medical Center,
	10.25 - 10.40	Coffee break
	12.00 - 13.00	Discussion, Q & A, closing remarks
		Lunch





Workshop

New trends in autoimmune diseases immunological profiling: Part I

Remotely via Zoom

27-28 October 2021

List of participants

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Workshop

New trends in autoimmune diseases immunological profiling: Part II

Rīga Stradiņš University, Institute of Microbiology and Virology 5 Rātsupītes Street, Riga

16-17 July 2022

List of participants

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	In person					
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4.	Gentili Valentina	University of Ferrara	PhD, Asst. Prof.	Valentina.Gentili@unife.it		
5.	Gilburd Boris	Sheba Medical Center	PhD, MD	Boris.Gilburd@sheba.health.gov.il		

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