

COMMENTARY TO HABILITATION THESIS¹

This habilitation thesis summarizes my long-term research focused on immunophenotypic and functional characterization of leukocyte subpopulations in patients with primary immunodeficiencies and immunocompromised conditions. My work combines advanced multiparametric flow cytometry with functional assays and clinical correlations, aiming to elucidate mechanisms of immune dysregulation and their relevance for disease stratification and patient management.

B-cell differentiation and CVID heterogeneity

My early work focused on B-cell abnormalities in common variable immunodeficiency (CVID). In the study identifying naïve B-cell subsets defined by CD24 expression, we demonstrated that the naïve B-cell compartment is phenotypically heterogeneous and that specific follicular B-cell subsets (FO I and FO II) are differentially affected in CVID. The observed imbalance of naïve B-cell subsets and expansion of CD21^{low} B cells provided early evidence that disturbances in early peripheral B-cell differentiation contribute to CVID pathogenesis, (1).

To address the complexity of B-cell heterogeneity, we subsequently applied automated multidimensional analysis of polychromatic flow cytometry data. Using probability binning, we identified distinct patient clusters that closely reflected established clinical and immunological classifications, including the EUROclass system. This work demonstrated that data-driven analytical approaches can objectively capture biologically relevant immunophenotypic patterns and improve reproducibility of CVID stratification, (2).

Age-dependent immune remodeling and immunosenescence

Further studies addressed age-related evolution of immune abnormalities in CVID. By analyzing T- and B-lymphocyte subsets across different age groups, we showed that CVID is characterized by progressive, coordinated alterations in both adaptive immune compartments. These changes resembled accelerated immunosenescence, including depletion of naïve T cells, accumulation of late-differentiated effector subsets, and expansion of CD21^{low} B cells. Importantly, T-cell activation and differentiation status correlated with B-cell abnormalities, highlighting interconnected dysregulation of adaptive immunity, (3).

Comparative analysis of CVID and selective IgA deficiency (sIgAD) revealed overlapping but quantitatively distinct immune alterations. While CVID patients exhibited profound T- and B-cell dysregulation, sIgAD patients showed milder yet detectable shifts toward memory and effector T-cell phenotypes. These findings supported the concept of a disease spectrum and suggested subclinical immune remodeling in sIgAD, (4,5).

¹ The commentary must correspond to standard expectations in the field and must include a brief characteristic of the investigated matter, objectives of the work, employed methodologies, obtained results and, in case of co-authored works, a passage characterising the applicant's contribution in terms of both quality and content.

Defective immune regulation: regulatory B cells and T cells

A key part of my work addressed functional immune regulation in CVID. We demonstrated that regulatory B cells (CD24^{hi}CD38^{hi}) are functionally impaired in CVID despite preserved frequencies. Reduced IL-10 production by these cells resulted in defective suppression of multifunctional IFN- γ ⁺TNF- α ⁺ CD4⁺ T cells, providing direct evidence that impaired B-cell-mediated regulation contributes to Th1-skewed inflammation in CVID. This study established an important mechanistic link between B-cell dysfunction and T-cell-driven immune dysregulation, (6).

Neutrophils and innate immune dysregulation in CVID

My later research expanded toward innate immunity, particularly neutrophils. We demonstrated that CVID patients exhibit increased numbers of activated neutrophils and granulocytic myeloid-derived suppressor cells (gMDSCs) with potent ROS-dependent T-cell suppressive capacity. These findings revealed that immune dysregulation in CVID extends beyond adaptive immunity and involves active participation of innate immune cells, (7).

Subsequent studies characterized low-density neutrophil subpopulations in CVID, identifying both mature and immature subsets with impaired oxidative burst and increased expression of immunosuppressive markers such as PD-L1. Functional correlations with reduced T-cell IFN- γ production further supported their regulatory and immunosuppressive role. In parallel, elevated plasma levels of neutrophil activation markers elastase and myeloperoxidase provided in vivo evidence of chronic neutrophil activation associated with autoimmune and lymphoproliferative complications, (8,9).

Neutrophil heterogeneity in trauma-induced immune dysregulation

The most recent part of my work focuses on neutrophil heterogeneity in trauma patients. Using multiparametric flow cytometry, we identified distinct neutrophil subpopulations with divergent functional properties and demonstrated their dynamic remodeling during the post-traumatic course. Exhausted neutrophils exhibited profound functional impairment and correlated with metabolic stress and trauma severity, whereas immature and hypersegmented subsets reflected emergency granulopoiesis and immune regulation. This work highlights neutrophils as central regulators of trauma-induced immune dysregulation and provides clinically relevant insights into post-traumatic immunosuppression, (10).

Overall contribution

Collectively, the studies presented in this thesis form a coherent research program spanning adaptive and innate immunity, integrating immunophenotyping, functional assays, and clinical correlations. My work contributes to a deeper understanding of immune dysregulation in CVID and trauma and demonstrates how detailed leukocyte profiling can reveal clinically meaningful mechanisms relevant for patient stratification and future immunomonitoring approaches.

1. VLKOVA, Marcela, Eva FRONKOVA, Veronika KANDEROVA, Ales JANDA, Sarka

RUZICKOVA, Jiri LITZMAN, Anna SEDIVA and Tomas KALINA. Characterization of Lymphocyte Subsets in Patients with Common Variable Immunodeficiency Reveals Subsets of Naive Human B Cells Marked by CD24 Expression. Journal of Immunology [online]. 2010, 185(11), 6431–6438. ISSN 0022-1767. Available at: doi:10.4049/jimmunol.0903876

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
50	50	50	70

2. KALINA, Tomas, Jan STUCHLY, Ales JANDA, Ondrej HRUSAK, Sarka RUZICKOVA, Anna SEDIVA, Jiri LITZMAN and Marcela VLKOVA. Profiling of Polychromatic Flow Cytometry Data on B-Cells Reveals Patients' Clusters in Common Variable Immunodeficiency. Cytometry Part A [online]. 2009, 75(11), 902–909. ISSN 1552-4930. Available at: doi:10.1002/cyto.a.20801

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
30	50	30	40

3. VLKOVA, M., V. THON, M. SARFYOVA, L. BLAHA, A. SVOBODNIK, J. LOKAJ and J. LITZMAN. Age dependency and mutual relations in T and B lymphocyte abnormalities in common variable immunodeficiency patients. Clinical and Experimental Immunology [online]. 2006, 143(2), 373–379. ISSN 0009-9104. Available at: doi:10.1111/j.1365-2249.2006.02999.x

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
70	30	50	30

4. LITZMAN, J., M. VLKOVA, Z. PIKULOVA, D. STIKAROVSKA and J. LOKAJ. T and B lymphocyte subpopulations and activation/differentiation markers in patients with selective IgA deficiency. Clinical and Experimental Immunology [online]. 2007, 147(2), 249–254. ISSN 0009-9104. Available at: doi:10.1111/j.1365-2249.2006.03274.x

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
70	30	30	30

5. NECHVATALOVA, Jana, Tomas PAVLIK, Jiri LITZMAN and Marcela VLKOVA. Terminally differentiated memory T cells are increased in patients with common variable immunodeficiency and selective IgA deficiency. Central European Journal of Immunology [online]. 2017, 42(3), 244–251. ISSN 1644-4124. Available at: doi:10.5114/ceji.2017.70966

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
30	50	30	50

6. VLKOVA, Marcela, Olga TICHA, Jana NECHVATALOVA, Tomas KALINA, Jiri LITZMAN, Claudia MAURI and Paul A. BLAIR. Regulatory B cells in CVID patients fail to suppress multifunctional IFN- γ +TNF- α +CD4+ T cells differentiation. Clinical Immunology [online]. 2015, 160(2), 292–300. ISSN 1521-7035. Available at: doi:10.1016/j.clim.2015.06.013

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
70	30	50	30

7. VLKOVA, Marcela, Zita CHOVANCOVA, Jana NECHVATALOVA, Ashley Nicole CONNELLY, Marcus Darrell DAVIS, Peter SLANINA, Lucie TRAVNICKOVA, Marek LITZMAN, Tereza GRÝMOVA, Premysl SOUCEK, Tomas FREIBERGER, Jiri LITZMAN and Zdenek HEL. Neutrophil and Granulocytic Myeloid-Derived Suppressor Cell-Mediated T Cell Suppression Significantly Contributes to Immune Dysregulation in Common Variable Immunodeficiency Disorders. Journal of Immunology [online]. 2019, 202(1), 93–104. ISSN 1550-6606. Available at: doi:10.4049/jimmunol.1800102

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
70	30	50	20

8. SLANINA, Peter, Julie STICHOVA, Veronika BOSAKOVA, Iva Staniczkoa ZAMBO, Marcela Hortova KOHOUTKOVA, Petra LAZNICKOVA, Zita CHOVANCOVA, Jiri LITZMAN, Terezie PLUCAROVA, Jan FRIC and Marcela VLKOVA. Phenotype and oxidative burst of low-density neutrophil subpopulations are altered in common variable immunodeficiency patients. Cytometry Part B-Clinical Cytometry [online]. 2024, 106(2), 99–112. ISSN 1552-4957. Available at: doi:10.1002/cyto.b.22150

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
20	70	30	70

9. LITZMAN, Jiri, Zita CHOVANCOVA, Petr BEJDAK, Marek LITZMAN, Zdenek HEL and Marcela VLKOVA. Common variable immunodeficiency patients display elevated plasma levels of granulocyte activation markers elastase and myeloperoxidase. International Journal of Immunopathology and Pharmacology [online]. 2019, 33(Article 2058738419843381). ISSN 2058-7384. Available at: doi:10.1177/2058738419843381

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
30	70	30	70

10. VLKOVA, Marcela, Julie STICHOVA, Karolina SURA, Karolina DVORAKOVA, Vojtech KUNCICKY, Ioanna PAPTAEODOROU, Gabriela BLAZKOVA, Zuzana TOMASIKOVA, Kamila BENDICKOVA, Ludmila DOHNALKOVA, Alexandra MYTNIKOVA, Jan ZAK, Jan KOVARIK, Milan KRTICKA, Tomas TOMAS, Vladimir SRAMEK, Martin HELAN, Anna KOCURKOVA, Jan FRIC and Marcela HORTOVA KOHOUTKOVA. Pro-inflammatory role of neutrophils populations in trauma patients: monitoring neutrophil populations. Frontiers in Immunology [online]. 2025, 16(Article 1565606). ISSN 1664-3224. Available at: doi:10.3389/fimmu.2025.1565606

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
70	30	50	50