ImmunoTools *special* Award 2025

Inga Dudek, PhD-student

Supervisor: dr. hab Agnieszka Kwiatek

Faculty of Biology, University of Warsaw, Ilji Miecznikowa 1
St. 02-096 Warsaw, POLAND

Immunomodulating capacity of non-structural proteins of respiratory syncytial virus and their potential therapeutic application

The human respiratory syncytial virus (hRSV) is a major cause of acute respiratory tract infections globally, predominantly impacting infants, older adults, and immunocompromised individuals. The pathogen infiltrates the respiratory tract, infecting alveolar epithelial lung cells, residing monocytes, and dendritic cells. Infection is characterized by a significant elevation in pro-inflammatory agents, such as IL-6, IL-8 (CXCL8), CCL5, MCP-1, CCL20, IL-4, IL-9, IL-17, and TNF-α. The key to successful infection and replication of the hRSV genome are non-structural proteins (NSs), namely NS1 and NS2. They're exceptionally versatile agents of immune interference, with a primary focus on the extensively studied interferon alpha (IFN-α) signaling pathway.

In my PhD project, I am investigating how the non-structural proteins of hRSV modulate the immune response. These proteins are known to block the non-specific immune response, facilitating viral propagation. My research suggests that NS proteins may influence the immune response beyond the IFN signaling pathway. Gene expression analysis and flow cytometry on transgenic human lung epithelial lines expressing hRSV non-structural proteins (A549/ns1, A549/ns2) reveal differential expression of immune-related genes and significant cytokine secretion, including IL-6, TNF-α, and CXCL8.

NS2, in particular, demonstrates a notable immunoactivation potential, causing a nearly fivefold increase in CXCL8 expression and secretion compared to NS1. The pro-inflammatory cytokines detected suggest an immune system response leading to further cytokine production and secretion of other pro-inflammatory or signaling factors. These findings motivate the next phase of my research: exploring the use of hRSV NS2 as a transgene with immunoactivation and anticancer potential. This involves examining the immune response in terms of activation, migration, and differentiation of immune cells.

Recent promising results show that the THP-1 cell line differentiates into macrophages when exposed to supernatants from the transgenic lines (A549/ns1, A549/ns2). I aim to expand this research to determine the phenotypes expressed and their pro- or anti-inflammatory profiles, which is crucial since M1 and M2 macrophages have distinct functions

and roles in the immune system. M2 macrophages, for instance, are associated with metastasis and the "cold" tumor environment, making this distinction critical. The ImmunoTools special Award will significantly aid in this research.

I plan to identify different subpopulations of monocytes (classical, non-classical, and intermediate) after exposure to infected/transfected epithelial cells using surface markers such as CD14, CD16, CD36, and HLA-DR. Additionally, I will conduct control experiments with the THP-1 cell line, differentiating it into M1 and M2 macrophages using LPS and IFN-γ for M1, and IL-4 for M2. These subpopulations will be identified by surface markers CD86, CD80, and CD163.

In conclusion, my research aims to deepen our understanding of the immunomodulatory effects of hRSV non-structural proteins, particularly NS2, and explore their potential therapeutic applications in immunoactivation and anticancer strategies. The ImmunoTools special Award would provide essential support in advancing this promising area of study.

ImmunoTools *special* AWARD for **Inga Dudek** includes 10 reagents

FITC - conjugated anti-human CD16, CD80, IL-6

PE - conjugated anti-human CD14, IL-8

PerCP - conjugated anti-human HLA-DR

APC - conjugated anti-human CD11b, CD40, CD86

recombinant human cytokines: rh IL-4

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