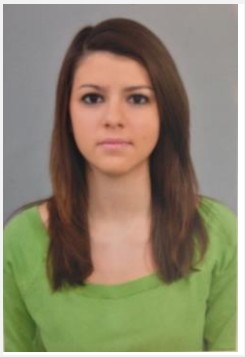
ImmunoTools *special* Award 2014



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The role of microvesicles released from cells during rhinovirus infection

Human rhinovirus (RV) infections are the most frequent cause of common colds which can worsen underlying lung diseases (e.g asthma, COPD). The quick nature of RV infection pathogenesis limits the benefits of antiviral drugs and to date there is no available therapeutic intervention with treatment focused specifically on symptom relief. Although RV is a nonenveloped virus we suspect that small membrane-enclosed microvesicles (MV's) released from RV infected cells modulate RV spread and cell-to-cell communication. MV's research is an expanding area and ranges from cancer to infectious disease but still many facts remain unclear. Our research is centred on characterising the role of MV's during rhinovirus infection. We will use in vitro models of RV infection and human samples from experimental RV infections to investigate how MV's modulate physiological responses by analysing their production and characterising their role in virus spreading.

To achieve our aim we have identified three major objectives. The most essential of which includes the characterisation of RV-induced MV's. To identify the parameters of interest (number, size, granularity, expression of phosphatidylserine, ICAM-1) flow cytometry analysis will be performed. The presence of signalling molecules (IL-6) and viral protein VP1 will be determined by ELISA and Western blotting experiments. PCR analysis will detect the RV genome in MV's. Another target of our project is to understand whether MV's modulate RV infections which will be accomplished through inhibition of MV's release and addition of exogenous MV's in cell cultures. Results will be obtained by flow cytometry, RT-PCR, Western blot and ELISA. To validate the in vitro data, nasal and lung washes of humans inoculated with RV will be analysed for MV content and number.

The ImmunoTools *special* AWARD box would be of tremendous value in allowing us to investigate MV's derived from RV-infected human cells and protein expression features of cells and MV's such as adhesion and development markers. We hope these experiments will provide clear details of the generation of MV's in response to RV infections with the ImmunoTool box giving us the opportunity to determine if RV derived MV's modulate the infection spread. Looking at the expression of phosphatidylserine and CD9, CD63 markers using annexin V and specific antibodies will enable us to exclude exosomes from our analyses and focus on MV's (see Figure1). These reagents provide a good platform of anti-human antibodies to begin our studies characterising MV release from infected cells. In addition, ELISA-set for the human cytokines IL-6 and IL-8 will enable measurement of MV's role in the modulation of RV infection responses.

Reagent	Application	Significance
FITC: anti-human CD9	Flow cytometry	Exosome marker
FITC: anti-human CD29	Flow cytometry	Adhesion marker
FITC: anti-human CD36	Flow cytometry	Adhesion marker
FITC: anti-human CD47	Flow cytometry	Exosome marker
FITC: anti-human CD95	Flow cytometry	Apoptotic marker
FITC: anti-human Control-IgG1*	Flow cytometry	Control
FITC: anti-human Control-IgG2a*	Flow cytometry	Control
FITC: anti-human Control-IgG2b*	Flow cytometry	Control
FITC: Annexin V	Flow cytometry	Microvesicle marker
PE: anti-human CD9	Flow cytometry	exosome marker
PE: anti-human CD44	Flow cytometry	Migration/adhesion marker
PE: Annexin V	Flow cytometry	Microvesicle marker
PE: anti-human Control IgG1*	Flow cytometry	Control
PE: anti-human Control IgG2a*	Flow cytometry	Control
PE: anti-human Control-IgG2b*	Flow cytometry	Control
Human IL-6 ELISA-set	ELISA	Inflammatory cytokine
Human IL-8 ELISA-set	ELISA	Inflammatory cytokine

*only required for specific conjugated antibodies of the same isotype
Figure 1: Reagents of interest supplied by ImmunoTools and their application

ImmunoTools *special* AWARD for **Simona Velkova** includes 22 reagents

FITC - conjugated anti-human CD9, CD29, CD36, CD47, CD54, CD95, Control-IgG1, Control-IgG2a, Control-IgG2b, Annexin V

PE - conjugated anti-human CD9, CD44, Annexin V, Control IgG1, Control IgG2a, Control-IgG2b

human IL-6 ELISA-set, human IL-8 ELISA-set (each 3 reagents)

DETAILS more AWARDS