# ImmunoTools *special* Award 2020

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## TRIM21 in Rhinovirus neutralization

### Background

Human rhinovirus (RV) causes more than half of common cold diseases which have a huge impact on the cost to society through medical treatment and missed days from school and work (Jacobs et al, 2013). RVs are non-enveloped positive sense RNA viruses with an icosahedral capsid made up of 60 copies of viral proteins 1-4 (VP1, VP2, VP3 and VP4). They are classified genetically (types A, B and C) and by entry into host cells (major and minor groups). Minor group RV (all type A) enter host cells through attachment to VLDL receptors while the major group (types A and B) enter cells through ICAM-1. Type C RVs are newly discovered and use another entry receptor known as CDHR3. To date there is no vaccine or treatment developed for RV infections and the only treatment available is palliative (Blaas et al, 2016). Despite years of research there is no protective vaccine approved yet, this is because of the emergence of more than 160 serotypes. Vaccine clinical trials have failed and not been performed since the 1970s because of the high antigenic variability of RV serotypes and difficulty in generating cross-serotype protection (Flather et al, 2018). More recently, researches have based vaccine studies on the selection of target antigens derived from the four-capsid proteins and how antibodies neutralize RVs. Studies have selected VP1 capsid protein as a vaccine immunogen and shown that cross serotype neutralising antibodies were generated (Edlmayr et al, 2011; McLean et al, 2012). Other studies using RV14 and RV2 serotypes have defined four neutralising immunogenic sites for antibodies – two on VP1 and one each on VP2 and VP3 (Sherry et al, 1986; Appleyard et al, 1990; Carey et al, 1992). These serotype-specific immunogenic sites have been termed NIm-1a, NIm-Ib, NIm-

II and NIm-III. Immunisations with a peptide mimicking NIm-II induced antibodies that were able to neutralize RV14 (Frances et al, 1987) and peptides corresponding to the VP1 ICAM1 binding site have induced antibodies that show cross protection of numerous RV strains (McCray et al, 1987). Our recent studies have demonstrated that antibodies induced by immunization with recombinant VP0 of RV16 capsid proteins and challenged with live RV16 were able to neutralize the virus through NIm-II on the VP2 region (Narean et al, 2019), Most antibodies are thought to neutralise RV by stabilising the capsid structure or blocking attachment to cells however, recent studies have identified a cytosolic protein TRIM21 that assists polyclonal antibodies in protecting against RVs (Watkinson et al, 2015). TRIM21 is able to bind to Fc region of antibodies attached to viruses when they enter cytosol, and recruit proteasomal degradation of the virions by generating free K63 polyubiquitin chains, through E3 ligase RING domain (Rakebrandt et al, 2014).

Aims: the aim of this project is to generate novel monoclonal antibodies that neutralise RV and to determine the role of TRIM21 in antibody neutralisation.

**1. Generation of monoclonal antibodies**

In this study we will try a novel approach to make monoclonal antibodies in vitro. We will isolate naïve murine splenocyte B lymphocytes and culture them for 7-14 days in vitro with combinations of RV peptide antigens and activation/survival factors. These culture conditions are hypothesised to mimic the in vivo conditions for antigen specific activation to produce desired antibodies. Culture supernatants will be screened for specific antibody production by ELISA. Cells from positive wells will be FACS-sorted into single wells for antibody V region RT-PCR. Recombinant monoclonal IgG will then be produced after V region cloning into antibody expression plasmids.

**2. Role of TRIM21 in RV neutralisation by antibodies**

We will study the neutralising antibodies and the role of TRIM21 in this process. We have an established RV16 and RV1B model of infection in vitro using HeLa and BEAS2b cells. RV-infected airway epithelial cells demonstrate cytopathic effect and release cytokines (IL-6 and IL-8) and interferons - we can determine the ability of antibodies to inhibit these processes. In this study we will examine cytokines

production induced by RV16 and RV1B infection in human epithelial cells with and without TRIM21 and determine the role of TRIM21 in RV neutralisation by antibodies.

For these studies the ImmunoTools reagents will be of great help.

ImmunoTools *special* AWARD for **Lila Touabi** includes 24 reagents:

FITC - conjugated anti-human :CD54, CD19, isotype control.

APC - conjugated anti-human: CD54, CD27, isotype control.

Human ELISA-sets: human IL-6, human IL-8, IFN- gamma

PE - conjugated anti-mouse: CD4, CD19, isotype control IgG2b.

APC - conjugated anti-mouse: isotype control IgG2b.

Recombinant mouse cytokines: IL-4, IL-7

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