ImmunoTools *special* Award 2014

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Human Cytomegalovirus-mediated immunosuppression: a viral protein targets immune cell functions

Infection with human cytomegalovirus (HCMV) can cause a severe illness in individuals with weakened or immature immune systems such as newborn babies, AIDS patients and transplant recipients. The often serious consequences of acute HCMV infection can also be further complicated by dangerous secondary infections resulting from additional immunosuppression induced by the virus. Transplant recipients, for example, suffer from an increased burden of secondary bacterial and fungal infections following acute HCMV infection. The mechanisms and viral genes involved in HCMV immunosuppression have not yet all been identified. Progress in this area promises to bring advances in HCMV treatment and also greater understanding of immune system function.

We showed recently that the previously uncharacterised HCMV protein pUL11 interacts specifically with the lymphocyte surface protein CD45 on all lymphocyte cell types and influences T cell functions, which is intriguing as it may shed new light on the mechanism of CD45 regulation, as well as providing an insight into HCMV pathogenesis (Gabaev I *et al.,* The human cytomegalovirus UL11 protein interacts with the receptor tyrosine phosphatase CD45, resulting in functional paralysis of T cells. PLoS Pathog 2011; 7(12):e1002432).

CD45 is expressed on the surface of all lymphocyte subtypes, e.g. T cells, B cells and NK cells. It has important roles in all cell types but has mostly been studied in T cells where it is a key functional regulator. In the absence of CD45, T cells cannot proliferate in response to incoming signals and severe combined immune deficiency (SCID) occurs. In Natural Killer cells cytokine and chemokine signalling is dramatically reduced in the absence of CD45.

In T cells, signals arriving at the T cell receptor (TCR) leading to T cell activation and proliferation are passed onwards by the Lck kinase, which must first be primed by dephosphorylation. As CD45 is the only phosphatase known to act on Lck, the action of CD45 is essential for setting the threshold at which incoming signals are transduced into effects.

The means by which CD45 activity is controlled remains disputed. The existence of a specific extracellular regulatory ligand for CD45 has long been postulated, but the many searches have so far been fruitless. With the HCMV protein pUL11 we

identified the first known specific CD45 ligand and also showed that T cell functions that are highly suggestive of effects on CD45 signalling seem to be affected by the interaction with pUL11. We showed a reduction in TCR dependent T cell proliferation in T cells treated with pUL11 and also that pUL11 treatment inhibits the cascade of tyrosine phosphorylation produced in T cells stimulated with an anti-TCR antibody.

We now aim to determine which of the many CD45 mediated functions are regulated by pUL11. To achieve this we will characterise the effects of pUL11 on different immune cell functions in more detail.

The ImmunoTools reagents would enable us to study the effects of pUL11 on CD4 and CD8 T cell subsets and to begin investigations in NK cells. We would initially investigate activation, proliferation, cytokine secretion and cell death. Using ImmunoTools cytokines and labelled antibodies we could stimulate lymphocytes and perform flow cytometry experiments and ELISAs to identify and detect functional changes in the various lymphocyte subsets.

CD45 is recognised as an important therapeutic target, with the potential to influence many diseases, particularly in the areas of autoimmunity, immunodeficiency and haematological malignancies. A means of regulating CD45 activity would therefore be of great interest in many fields.

With this project we hope to shed light on how a key modulator of lymphocyte function is itself regulated, which may lead to a better understanding of a mechanism by which HCMV infection attacks immune functions in vulnerable patients and also to the development of new therapies against immune system dysfunction.

ImmunoTools *special* AWARD for **Jasmin Zischke** includes 25 reagents

FITC - conjugated anti-human CD3, CD4, CD25, CD45RA, CD45RB, CD56, CD69, IL-6, Control-IgG1, Control-IgG2a, Annexin V,

PE - conjugated anti-human CD4, CD11c,

PerCP - conjugated anti-human CD45,

APC -conjugated anti-human CD3, CD8, CD56, CD62L, CD69, IL-6,

recombinant human cytokines: rh IL-6, rh IL-10,

human IL-6 ELISA-set, (with 3 reagents) DETAILS more AWARDS