ImmunoTools *special* Award 2014

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Evaluation of HCMV vaccine properties in humanized mouse model

Human cytomegalovirus (HCMV) is a cause of widespread infectious disease that often leads to severe medical complications in immunocompromised individuals and immunologically immature neonates. During evolution with its host, cytomegaloviruses (CMVs) have developed numerous immune evasion mechanisms which enable them to establish lifelong infection with occasional reactivation events. Regardless of such an effective immune-evasion, CMV infection induces surprisingly strong T cell response what makes this virus a good vaccine candidate. NKG2D is a NK cell activating and T cell co-stimulatory receptor and is particularly important in the control of viral infections. The importance of NKG2D in viral immune control is underscored by the fact that CMVs encode several immune evasion mechanisms directed against NKG2D resulting in down-regulation of its ligands from the surface of the infected cell [1]. Recently, our group generated a recombinant mouse cytomegalovirus (MCMV) expressing NKG2D ligand RAE1y in place of viral immune-evasion gene targeting Rae1γ (*m152*) [2]. MCMV-RAE1y recombinant virus, in spite of its severe attenuation, provided excellent CD8 T cell response which ensured long-term protection following challenge infection and even protection against vertical transmission of the virus. The protective capacity of MCMV-RAE1y was further confirmed in experiments with recombinant virus carrying CD8 T cell epitope of *Listeria monocytogenes* (LM), which provided protection against subsequent challenge infection with *LM* [3]. How can this severely attenuated virus vector induce strong immune response is not completely understood but preliminary results suggest a complex signaling network mediated by NK cells which leads to modulation of CD8 response.

We would now like to apply the principle employed in MCMV to HCMV system and explore NK and T cell responses to recombinant HCMV viruses. To achieve this we have constructed HCMV recombinant viruses carrying deletion of certain immune evasion gene and expressing human NKG2D ligand. Research of immunological response in context of HCMV infection is significantly limited by high species specificity of HCMV. To overcome this issue we are using severely immunodeficient mice strain engrafted with human immune cells (humanized mice). NOD/Shi-scid/IL-2Rγnull HLA-A2 (NOG HLA A2) mice receive CD34 positive cells derived from human fetal liver as newborns and are screened three months post engraftment by flow cytometry to determine reconstitution of respective populations of human immune system using anti-human and anti-mouse CD45 antibodies. Individual subsets are identified using anti CD3, CD4, CD8, CD19, CD56 and CD33 antibodies.

Another challenge we are confronting is the infection of humanized mice given that *in vivo* studies on HCMV tropism showed that cells of myeloid origin circulating in the peripheral blood are susceptible to the HCMC infection but the development of myeloid in humanized mice is not efficient enough to provide adequate number of cells to support HCMV infection, thus we will expand and differentiate CD34 cells *in vitro* into autologous dendritic cells. After incubation of CD34 cells with combination of cytokines previously shown to drive differentiation of stem cells towards dendritic cell phenotype (TPO, SCF, Flt3L, GM-CSF, IL-4, IL-3 and TNF-α), we will thoroughly characterize generated cells using specific antibodies against human HLA-DR, CD1a, CD11c and DC-SIGN to confirm linage commitment. To assess up-regulation of co-stimulatory molecules and maturation capacity, expression of CD83, CD38, CD40, CD80 and CD86 will be determined after stimulation with LPS. Subsequently, dendritic cells will be infected with recombinant HCMV viruses and injected into humanized mice. We will follow viral load *in vivo*, as well as phenotype and activation status of NK cells using anti CD11b, CD27, CD69 and KLRG1. Additionally, T cell compartment will be characterized based on CD45RA, CCR7 and CD62L expression to separate naïve T cells from effector, effector memory and central memory.

These experiments will increase our knowledge about the nature of interaction between NK and T cells in the context of HCMV infection. Furthermore, detailed characterization of immunological response against various recombinant viruses could result in a live HCMV vector capable of inducing robust and enduring T cell responses. Therefore, receiving ImmunoTools *special* Award in form of specific antibodies for flow cytometry and recombinant human cytokines for *in vitro* studies would be of great benefit to our experiments.

1. Lenac T, Arapović J, Traven L, Krmpotić A, Jonjić S (2008) Murine cytomegalovirus regulation of NKG2D ligands. Med Microbiol Immunol 197: 159-166.
2. Slavuljica I, Busche A, Babić M, Mitrović M, Gašparović I, et al. (2010) Recombinant mouse cytomegalovirus expressing a ligand for the NKG2D receptor is attenuated and has improved vaccine properties. J Clin Invest 120: 4532-4545
3. Trsan T, Busche A, Abram M, Wensveen FM, Lemmermann NA, et al. (2013) Superior induction and maintenance of protective CD8 T cells in mice infected with mouse cytomegalovirus vector expressing RAE-1γ. Proc Natl Acad Sci U S A 110: 16550-16555.

ImmunoTools *special* AWARD for **Lea Hiršl** includes 25 reagents

FITC - conjugated anti-human CD1a, CD11b, CD33, CD45RA, CD38, CD40, CD69,

PE - conjugated anti-human CD3, CD19, CD14,

PerCP - conjugated anti-human CD3, CD8,

APC - conjugated anti-human CD3, CD27, CD11c,

recombinant human cytokines: rh FGF-b / FGF-2, rh Flt3L /CD135, rh GM-CSF, IL-2, rh IL-3, rh IL-4, rh SCF, rh TNFα, rh TPO, rh IFNgamma DETAILS more AWARDS