C4 Frank Neipel & Hans-Martin Jäck
Recombinant MHV-68 as a model system for KSHV-vaccine development

State of the art and own previous work. Almost all tumor cells of KSHV-associated malignancies harbor the viral genome in a latent state. However, the development of KS-lesions is associated with an increase of antibodies against lytic KSHV-proteins indicating that lytic replication precedes KS development. Nevertheless, antiviral drugs currently available against KSHV and other herpesviruses are not effective against KSHV-associated malignancies. Passive and/or active immunization against KSHV would thus be important for people at risk for KS due to immunosuppression or living in areas of high KSHV prevalence. Despite many efforts, the only effective vaccine available today against a human herpesvirus is the Oka vaccine against varicella-zoster virus (VZV). A life-attenuated vaccine was usually seen as the only way to achieve this goal. But vaccination with a potentially oncogenic herpesvirus raises major safety concerns due to the inherent ability of lifelong persistence. However, this view has been challenged in the past few years through lessons learned from the highly effective vaccine against human papillomaviruses (HPV) and also from studies on immunity against human cytomegalovirus (HCMV): it turned out the antibodies against structural virion proteins – capsid components in the case of HPV and viral membrane glycoproteins in the case of HCMV - are sufficient to obtain sterilizing immunity. The group of Frank Neipel studies function and immunogenicity of KSHV glycoproteins since the discovery of the virus. We identified the KSHV-specific glycoprotein gpK8.1 (Raab et al., 1998) and were able to show that it mediates virion attachment by binding to heparansulfate (Birkmann et al., 2001). In addition, we identified two cellular receptors for the glycoprotein complex gH/gL (Hahn et al., 2012; Hahn et al., 2009) and were able to show that soluble variants of the cellular protein EphA2 efficiently neutralize KSHV in cell culture (Hahn et al., 2012). Although KSHV codes for more than 80 proteins, the antibody response in KSHV infected patients is essentially limited to only two antigens, namely gpK8.1 and the latent nuclear antigen LANA (Pellett et al., 2003). Moderate neutralizing activity is detectable in the sera of KSHV infected individuals (Dialyna et al., 2004). Although the targets of these neutralizing antibodies have not been identified, gpK8.1 is the most likely candidate. KSHV is highly species specific and unable to replicate in non-primate hosts. The closest relative to KSHV in rodents is MHV68. In MHV-68, gp150 is the positional and functional analog to gpK8.1. gp150 has already been shown to be the target of neutralizing antibodies (Stewart et al., 1999) although it is dispensable for infection (Ruiss et al., 2012). The group of Hans-Martin concentrates on molecular aspects of the humoral immune response and engineered a new mouse strain, the “Mighty mouse”. All murine antibody gene sequences have been replaced in the “Mighty mouse” with the synthetic human gene segments. Thus the “Mighty Mouse” produces human antibodies and replicates the antibody-generating capacity of human beings. During the first PhD project we could show that the neutralizing response of KS-patient sera is directed against gpK8.1 and that neutralizing antibodies can be generated against gpK8.1 by immunizing mice. A first series of monoclonal antibodies could also be generated using the “mighty mouse”. Analysis of these antibodies for neutralizing capacity is currently on the way.

PhD project. As antibodies that neutralize virus-infection in cell culture are not necessarily efficient in vivo, an animal model will be required for vaccine development The 2nd PhD project will focus on the further development of a mouse model based on recombinant MHV-68.. We will therefore develop a model system based on MHV-68. Interestingly, both gp150 and gpK8.1 are dispensable for infection. This implies that the core fusion machinery comprising gH/gL and gB is functional without a specific interaction with gpK8.1 or gp150. It should thus be possible to replace the ectodomain of gp150 in MHV-68 with the ectodomain of gpK8.1. This will be done be mutagenizing an already available BAC-clone of MHV-68. After testing the immune response of mice immunized against gpK8.1 they will be challenged with the “KSHV-ized” MHV-68 and the infection process will be monitored to identify neutralizing immune responses. In addition, immunizations against other viral glycoproteins – especially using glycoprotein B – will done and their neutralizing capacity will be compared with antibodies against gpK8.1

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Collaborations within the Research Training Network

The generation of recombinant MHV68 will be done in collaboration with B3. The characterization of antibody responses against KSHV and the development of immunization strategies will be performed in collaboration with B1, B2, C3 and C5. Monoclonal antibodies will be generated and characterized in collaboration with C3.