**Application for a project in the PhD program of the International Graduate School in Molecular Medicine Ulm.**

**1.1. Applicant**
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**1.2 Topic** (#4 host-microbe interactions)
**Characterization of hepatitis C core protein-mediated immune modulatory effects in antigen presenting cells targeted by DNA-based vaccines and in antigen-expressing transgenic hepatocytes.**

**1.3 Summary**
Hepatitis C virus (HCV) leads to chronic hepatitis in the majority of infected individuals. Chronic HCV patients frequently establish liver fibrosis and hepatocellular carcinoma. To establish viral persistence HCV has to evade the host`s cellular and humoral immune system but the mechanisms are not well understood. It has been shown in patients and murine model systems that the HCV core protein (HCV-C) has a strong immune modulatory impact e.g., on dendritic cell functions, interferon signalling or cell surface expression of MHC class-I molecules. The proposal aims to elucidate systemic and intrahepatic regulatory effects of HCV-C that drive down-regulation of anti-viral immune responses.

We have established a HCV transgenic (tg) mouse model (1) in our lab. These mice express the HCV-C antigen in hepatoctes. Interestingly, DNA- and protein-based vaccines did not induce HCV-C specific CD8 T-cell responses in control (H-$2^b$) B6 and HCV-tg mice. It is unknown whether the missing CD8 T-cell response in B6 mice depends on an inefficient priming and maintenance of HCV-C specific CD8 T-cells or on the absence of H-$2^b$ binding epitopes in the HCV-C protein. This mouse model is very attractive to study the regulatory effects of HCV-C *in vivo*: (i) during the induction of CD8 T-cell responses directed against other antigens (e.g., OVA or hepatitis B antigens) by DNA-based vaccination and (ii) during the cross-talk of hepatocytes, co-expressing HCV-C and other antigens and viral genomes (e.g., HCV/HBV tg hepatocytes), with HBV-specific CD8 T-cells.

In a DFG funded project (DFG Schi505/5-1) we are currently analyzing 1.4HBV-$S^{mut}$ tg mice that harbour a replicating HBV genome, produce HBV core, precore and endogenous (but not secreted) surface antigens in the liver. We showed that HBV-core (but not HBV-surface) antigen-specific DNA vaccines elicit anti viral CD8 T-cell responses in 1.4HBV-$S^{mut}$ tg mice (2). HBV-core-specific CD8 T-cells specifically recognize 1.4HBV-$S^{mut}$ tg hepatocytes and efficiently (but transiently) suppress HBV replication. In the proposed project we will design immunization experiments with HCV-C and HBV-surface or HBV-core co-expressing vectors and single antigen expressing vectors and compare the frequencies and cytokine-secretion (e.g., IFN$\gamma$) of HBV-specific CD8 T-cell responses. This will allow us to detect immune regulatory interactions of HCV-C in antigen presenting cells targeted by intramuscular vector injection. Furthermore, we will breed HCV/1.4HBV-$S^{mut}$ tg double tg mice and analyze whether direct immunization with HBV-core DNA or adoptive transfer of HBV-core-specific CD8 T-cells differ in 1.4HBV-$S^{mut}$ tg and HCV/1.4HBV-$S^{mut}$ tg mice. This will allow us to detect immune regulatory interactions between HCV-C and HBV-specific antigen presentation and viral replication in hepatocytes. The results of these studies may contribute to understand HCV-C specific immune regulatory functions *in vivo*. We further expect to get new informations on HBV / HCV interactions in hepatocytes that operate in chronic HBV/HCV co-infected patients.

The project will be performed in cooperation with Prof. Dr. Alfredo Alberti and Prof. Dr. Giorgio Palù (University of Padova) and Prof. Dr. Thomas Mertens (University of Ulm). Prof. Thomas Seufferlein (Head of Internal Medicine I) has assured his support.

1) Alonzi T, Agrati C, Costabile B, Cicchini C, Amicone L, Cavallari C, Rocca CD, Folgori A, Fipaldini C, Poccia F, Monica NL, Tripodi M. (2004) Steatosis and intrahepatic lymphocyte recruitment in hepatitis C virus transgenic mice. J Gen Virol. 85:1509-20.
2) Riedl P, Wieland A, Lamberth K, Buus S, Lemonnier F, Reifenberg K, Reimann J, Schirmbeck R. (2009) Elimination of immunodominant epitopes from multispecific DNA-based vaccines allows induction of CD8 T cells that have a striking antiviral potential. J Immunol. 183(1):370-80.