Project I

Obesity and insulin type 2 Diabetes mellitus (T2DM) are intimately linked to an increased risk and disadvantageous clinical outcome of mycobacterial infections. The immunological basis for this phenomenon is not yet clearly understood. The mechanisms associated with DM potentially leading to increased susceptibility to mycobacterial infections and disadvantageous clinical outcome include factors that are instantaneously related to hyperglycemia and cellular insulinopenia. These factors may lead to impaired macrophage and lymphocyte functions, such as chemotaxis, phagocytosis, activation, as well as antigen presentation, leading to diminished ability to contain and control the mycobacteria. In this work, we tried to explore the immunological basis of the alliance between Diabetes and mycobacterial infections. To this end we used two different mouse models to distinguish metabolic and non-metabolic factors that would potentially impact on the immune response to mycobacterial infections. One hallmark of T2DM is the down-regulation of insulin receptor (IR), which is in addition to conventional insulin target tissues, also expressed on macrophages (MΦ), the host cells of mycobacteria. We therefore investigated the immunological outcome of macrophage-autonomous defense mechanisms against *M. avium* infection *in vitro* as well as the outcome of *in vivo* infections in a mouse model with a myeloid cell-specific IR knock out ($IR^{\Delta myel}$-mice). These mice are non-diabetic and they have been shown to exhibit decreased concentrations of circulating TNF-α, a key cytokine in macrophage defense against mycobacteria. The second mouse model, (db/db mouse), has a mutation of the long-isoform leptin receptor gene and is a widely used model for obesity and T2DM. In both mouse models the killing capacity of BMDMs towards *in vitro M. avium* infection was assessed, as well as the outcome of long-term *in vivo* infection (2 months) and short-term infection (4 / 8 days). To this end we evaluated organ bacterial burden, granuloma formation and serum cytokine levels during infection. Overall there was no difference between ctrl mice and $IR^{\Delta myel}$-mice with regard to the *in vivo* and *in vitro* parameters evaluated, except of significantly more granuloma in the liver of $IR^{\Delta myel}$-mice after 2 month of *M. avium* infection. However, differences in granuloma counts did not affect bacterial burden and therefore cannot give an indication on bacterial control and are consequently negligible. Thus, in all probability myeloid-cell type specific-autonomous IR mediated signaling is dispensable to combat mycobacteria. Also for the db/db mice there was no difference in *in vitro*

killing capacity and cytokine secretion of BMDMs. As described in the literature we observed that db/db mice have obesity-associated atrophy of the spleen and lungs. For long-term infection of 2 months this was associated with significantly lower bacterial burden and granuloma counts in the spleens of db/db mice in comparison to ctrl mice. In addition to spleen and lung atrophy observed in older mice (≈ 2 months of age) we observed that younger db/db mice (up to 8 days of age) have enlarged livers (absolute liver weight) in comparison to controls, which is also in accordance with the literature. In contrast to observations made after long-term infection liver bacterial burden after short-term infection was independent from organ size. After 4 days of infection db/db mice exhibited higher absolute, but not relative CFUs in their larger livers. After 8 days of infection absolute liver CFUs were comparable, while relative liver CFUs of db/db mice were lower in comparison to that of controls. As bacterial burden of organs correlated with weight of spleens but not of livers, in all probability other parameters such as cellular composition of organ tissues that inevitably contribute to organ weight are important for establishment or clearing of infection. This has to be, subject of further investigations. In conclusion, neither IR deficiency on myeloid cells in $IR^{\Delta myel}$-mice nor the metabolic changes contributing to Diabetes in db/db mice had a negative impact on the course and outcome of *M. avium* infection in mice. Thus these investigations could not unravel the causative factors for the alliance of Diabetes mellitus and mycobacterial infections.

Project II

The development of new and effective vaccines to prevent tuberculosis is a declared aim of the WHO. In this study we set out to develop a new vector-based vaccine candidate. To this end we designed recombinant Adeno-Associated viral based vectors encoding the mycobacterial antigen Ag85A. First we tested two AAV serotypes (AAV2:Ag85A and AAV5:Ag85A) in a prime-boost vaccination scheme in Balb/c mice for both, induction of humoral and cellular immune responses. A single i.m. injection in mice was well tolerated and led to a strong and sustained humoral immune response towards Ag85A. Important, mice immunized with AAV2 did not produce antibodies against the AAV5 capsid. This absence of cross-reactive antibodies enables to operate the prime-boost vaccination strategy with these two different rAAV serotypes. AAV2 prime- followed by AAV5 boost vaccination induced a specific anti-Ag85A cellular immune response in cultivated splenocytes after re-stimulation with Ag85 protein, as determined by quantification of cytokine secretion. While splenocytes from mice immunized with the very same vaccination scheme with rAAV vectors encoding for the GFP protein (AAV2:GFP and AAV5:GFP), used as negative control, did not induce Ag85A specific cellular immune response following Ag85 re-stimulation.

Next, in order to increase immunogenicity of AAV vectors, we made use of the AAV2 capsid as scaffold for direct antigen Ag85A display. We hypothesized that combining direct antigen display on the AAV capsid followed by subsequent vector-mediated antigen over-expression, would induce a prime-boost effect upon a single-shot vaccination. In order to investigate, whether this hypothesis holds true we initiated a proof-of-principle study. In deed a single-shot with the capsid-modified “prime-boost” vector Ag85A-AAV:Ag85A, induced antigen-specific humoral immune responses significantly faster and with higher IgG antibody levels of higher avidity than the conventional vector AAV:Ag85A or the recombinant Ag85A protein, administered subcutaneously. Furthermore, Ag85A incorporation into the AAV capsid alone (Ag85A-AAV:GFP) induced higher IgG specific antibody levels than administration of the protein itself, even though Ag85A concentration in Ag85A-AAV:GFP vector preparations was remarkably lower than the concentration of the pure protein. To further investigate the contribution of capsid-modification as such, we checked for memory recall responses as a measure of antigen specific immunogenicity. Upon subcutaneous booster vaccination with rAg85A, applied 3 months post prime vaccination, IgG titers obtained 2 weeks post boost, were significantly higher in serum of all mice previously primed with capsid-modified vectors than in mice primed with AAV:Ag85A. The most impressive

memory/recall response was elicited in mice treated with Ag85A-AAV:GFP, the vector solely presenting Ag85A as antigen on the capsid. Here, compared to baseline, antibody titers increased about 16-fold compared to mice initially vaccinated with rAg85A (only 4-fold). Thus, antigen incorporation into AAV capsids augments antigen specific immunogenicity and is by itself sufficient to induce a memory/recall response.

Our findings demonstrate that combining antigen incorporation into the AAV capsid with over-expression of the antigen after cell transduction dramatically enhances the antigenic potential of AAV-based vaccines. Furthermore incorporation of antigens into the AAV capsid is conceivable for a variety of applications such as vaccination with multiple antigens using a single construct. Overall this work demonstrates the potency of conventional as well as capsid-modified AAV vectors in inducing of antigen-specific adaptive immune responses.