Around 400 million people are currently suffering from diabetes and it is estimated that this number will increase to almost 600 million by the year 2035. Due to its chronic nature and associated severe long term pathology, diabetes is one of the most cost-intensive diseases. New treatment regimens that prevent diabetes development, stop its progression or even reverse it, will have a huge impact on the welfare of people and reduce health care costs.

Type 1 Diabetes is an autoimmune disease which is caused by the destruction of insulin producing pancreatic β-islet cells by autoreactive T cells and accounts for 5-10% of all diabetes cases. Type 2 Diabetes represents 90% of all diabetes cases and occurs due to the development of insulin resistance. Obesity is one major etiological factor for the development of insulin resistance, which results in adipose tissue inflammation and subsequent changes in its cellular composition and the development of insulin resistance. Reduction of inflammation that occurs in the pancreas and in the adipose tissue may therefore prevent or delay the onset of Type 1 Diabetes and improve insulin sensitivity, respectively.

Over the past decades, the incidence of Type 1 Diabetes and other autoimmune diseases increased at a rate in industrialized countries that suggests that environmental factors contribute to this recent increase. One such environmental factor is the coinciding loss of parasitic worm (helminth) infections in developed countries. Parasitic helminths are potent modulators of their host's immune system and establish regulatory, anti-inflammatory immune responses which enable their long term survival within their hosts, but also affect immune responses to bystander antigens. Loss of helminth infections may hereby also contribute to the recent rise of Type 2 Diabetes, by suppressing pro-inflammatory immune responses that are associated with the development of insulin resistance.

Using the nonobese diabetic (NOD) mouse model for Type 1 Diabetes the aim of the IRG grant was to identify which helminth-induced regulatory mechanisms are required to mediate protection against the onset of Type 1 Diabetes and establish a therapy which provides protection after insulitis occurred, i.e. destruction of β-islet cells started. Furthermore, it was investigated whether parasitic helminths and their antigens improve insulin sensitivity in diet-induced obesity mice and which protective mechanisms may be involved.

Results from our study indicate that infections with the filarial nematode *Litomosoides sigmodontis* prevent the onset of Type 1 Diabetes in NOD mice in a manner that was dependent on the anti-inflammatory cytokine TGFβ, but was independent of anti-inflammatory IL-10 and regulatory T cell responses (Hübner et al. 2012, J Immunol). Furthermore, we demonstrate that administration of *L. sigmodontis* adult worm antigen (LsAg) in combination with pro-insulin prevents diabetes onset in 10-week old NOD mice after the development of insulitis, a time point when neither infection with *L. sigmodontis* nor administration of LsAg or pro-insulin alone protected (Fig. 1). Animals that received the combination therapy had significantly increased frequencies of regulatory T cells within the pancreas and pancreatic lymph nodes and had significantly increased numbers of healthy, non-infiltrated pancreatic islets. This suggests that the efficacy of antigen-specific therapies like the administration of intranasal pro-insulin can be further improved by combination with immunoregulatory helminth-derived antigens. The potential of such combination therapies for human therapy should be further investigated, as it could delay the onset or progression of Type 1 Diabetes in humans and reduce long term pathology.

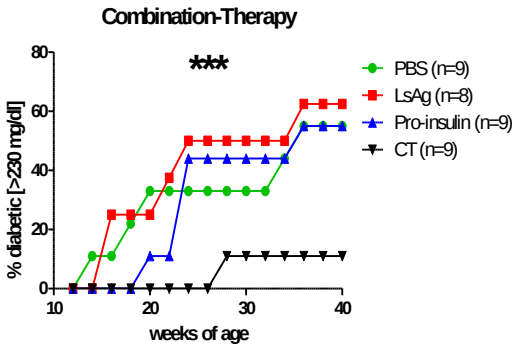

Figure 1. Combination of LsAg and proinsulin protects 10-week old female NOD mice from type 1 diabetes onset. Frequency of diabetic NOD mice after treatment with LsAg and pro-insulin or both (CT), and PBS controls. ***p<0.001.

In regard to diet-induced insulin resistance, infection with *L. sigmodontis* and treatment with LsAg improved glucose tolerance in diet-induced obesity mice (Fig. 2). Obesity caused changes in the cellular composition of the adipose tissue, including loss of regulatory cell types like alternatively activated macrophages and regulatory T cells as well as eosinophils, and increased accumulation of B cells and pro-inflammatory, classically activated macrophages were reversed by *L. sigmodontis* infection and LsAg treatment.

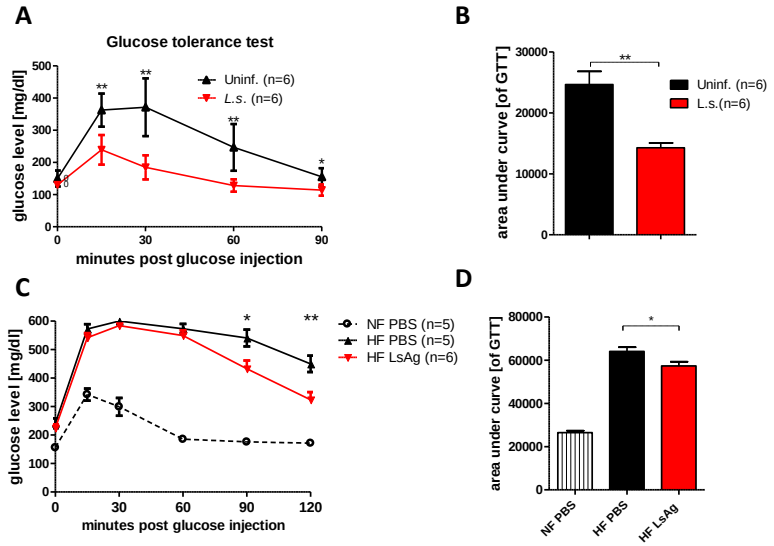

Figure 2. Infection with *L. sigmodontis* and two weeks of daily LsAg injections improve glucose tolerance in diet-induced obesity mice. *A, C,* Blood glucose levels over time during a glucose tolerance test and *B, D,* area under the curve obtained from the glucose tolerance tests. Animals were either infected with *L. sigmodontis (A, B),* or treated with LsAg or PBS (*C; D*) starting at 4 and 12 weeks after high fat (HF) diet, respectively. Analyses were performed at 12 and 14 weeks of HF diet, respectively. *p < 0.05; **p < 0.01.

Those treatments of diet-induced obesity mice restored a cellular composition within the adipose tissue that resembled lean mice. This included significantly increased regulatory T cell, alternatively, activated macrophage and eosinophil frequencies, whereas frequencies of B2 cells and classically activated macrophages were reduced. Induction of eosinophils was essential as *L. sigmodontis* mediated improvement of glucose tolerance in diet-induced obesity mice was not given in eosinophil-deficient dblGATA mice. Gene expression analysis from epididymal adipose tissue of repeated LsAg treated animals further revealed a significantly increased expression of genes that promote insulin sensitivity and a reduced expression of genes that are associated with adipose tissue inflammation. LsAg administrations further induced the browning of subcutaneous adipose tissue. Browning of adipose tissue presents a novel approach to treat diet-induced obesity and insulin resistance, as it increase energy expenditure by dissipation of energy in the form of heat. Thus, administrations of helminth antigens do not only reduce diet-induced adipose tissue inflammation, but induce gene expression that improve insulin signaling and increase energy expenditure via the browning of subcutaneous adipose tissue. Helminth-derived antigens present therefore potential novel therapies to treat obesity and obesity-associated metabolic diseases like insulin resistance and Type 2 Diabetes via the induction of several protective mechanisms.

In summary, we were able to show that filarial infection and filarial antigens can protect against the onset of Type 1 Diabetes and ameliorate diet-induced insulin resistance. Identification of those regulatory mechanisms and identification of helminth-derived products that induce those mechanisms may provide a powerful tool to combat autoimmune and metabolic diseases which represent a growing public health concern.