Metab-Immune analysis of the Non-obese diabetic mouse

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Akademisk avhandling

som med vederbörligt tillstånd av Rektor vid Umeå universitet för avläggande av filosofie/medicine doktorsexamen framläggs till offentligt förvar i Salens namn eller beteckning, byggnad 6D, A5_RO, Fredagen den 13 Maj, kl. 13:00.
Avhandlingen kommer att förvaras på engelska.

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Abstract

Type 1A diabetes mellitus or T1D is a chronic disease characterized by T cell mediated destruction of the insulin producing β cells in the islets of Langerhans. The classical symptoms include high glucose levels in urine and blood, polyuria, and polydipsia. Complications associated with T1D include blindness, amputations, and end-stage renal disease, and premature death. The non-obese diabetic (NOD) mouse, first described in 1980, is widely used as a model organism for T1D. T1D disease in the NOD mouse shares a number of similarities to human T1D including dependence on genetic and environmental factors. More than 30 disease associated gene regions or loci (termed insulin dependent diabetes, or Idd, loci) have been associated with T1D development in NOD. For some of these Idds, the corresponding region in human has been linked to the development of T1D in human.

T1D, both in humans and mice, is recognized as a T cell mediated disease. However, many studies have shown the importance of both the metabolome and the immune system in the pathogenesis of the disease. Appearance of autoantibodies in the serum of patients is the first sign of pathogenesis. However, molecular and cellular events precede the immune attack on the β-cell immunity. It has been shown that patients who developed T1D have an altered metabolome prior to the appearance of autoantibodies. Although much is known about the pathogenesis of T1D, the contribution of the environment/immune factors triggering the disease is still to be revealed.

In the present study both metabolic and immune deviations observed in the NOD mouse was analyzed. Serum metabolome analysis of the NOD mouse revealed striking resemblance to the human metabolic profile, with many metabolites in the TCA cycle significantly different from the non-diabetic control B6 mice. In addition, an increased level of glutamic acid was of the most distinguishing metabolite. A detailed bioinformatics analysis revealed various genes/enzymes to be present in the Idd regions. Compared to B6 mice, many of the genes correlated to the metabolic pathways, showed single nucleotide polymorphism (SNP), which can eventually affect the functionality of the protein. A genetic analysis of the increased glutamic acid revealed several Idd regions to be involved in this phenotype. The regions mapped in the genetic analysis harbor important enzymes and transporters related to glutamic acid. In-vitro islet culture with glutamic acid led to increased beta cell death indicating a toxic role of glutamic acid specifically towards insulin producing beta cells.

In the analysis of the immune system, B cells from NOD mice, which are known to express high levels of TACI, were stimulated with APRIL, a TACI ligand. This resulted in enhanced plasma cell differentiation accompanied with increased class switching and IgG production. NOD mice have previously been shown to react vigorously to T-dependent antigens upon immunization. In this study we confirmed this as NOD mice showed an enhanced and prolonged immune response to hen egg lysozyme. Thus, serum IgG levels were significantly increased in the NOD mice and were predominantly of the IgG1 subtype. Immunofluorescence analysis revealed increased number of germinal centers in the NOD mice. Transfer of purified B and T cells from NOD to an immune deficient mouse could reproduce the original phenotype as seen in the NOD mice.

Collectively, this thesis has analyzed the metabolomics and immune deviations observed in the NOD mouse.

Keywords
Type 1 diabetes, B cells, Glutamic acid, NOD mouse, Metabolomics, Idd