All forms of diabetes are characterized by chronic hyperglycemia that is elevated levels of blood glucose. This imbalance in blood glucose level plays a central role in the development of diabetes specific microvascular pathologies such as nephropathy, retinopathy and neuropathy. The principal cause leading to diabetic complications is the non enzymatic modification of proteins known as glycation, which involves the series of reactions between a reducing sugar such as glucose, or a ketoaldehyde such as methylglyoxal (MG) and the amino groups of proteins known as glycation. Glycation eventually leads to formation of AGE modified proteins which exhibit altered structure and functionality from native proteins and even form aggregates. Such structurally altered glycated proteins present new immunological epitopes on their surface that are recognised by the host immune system as “no self” or “autoantigens” leading to generation of autoantibodies. The autoantibodies bind to modified antigens leading to formation of CICs. Autoimmune responses against modified self-antigens in the development of diabetic vascular complications represent a relatively unexplored concept. Comprehensive identification of novel antigens associated with CICs which are real time products of immune response, potentially could provide significant new mechanistic insight into the underlying disease process. Therefore, this thesis mainly focuses on identification, quantification and characterization of CIC associated glycated proteins acting as autoantigens using proteomic approaches. In addition the role of reactive immunization with CML AGE modified MSA were studied in normal mice.

Proteomic analysis of glycated proteins in CICs from clinical plasma

Elicitation of the immune response against AGE modified proteins generates autoantibodies directed against these modified proteins, eventually leading to formation of CICs. Hence with the objective of identification of glycated proteins acting as autoantigens we isolated CICs from clinical plasma samples from healthy control
(CON), prediabetes (IGT), newly diagnosed diabetes (NDD), diabetes (DM) and diabetes with microalbuminuria (DM-MIC). CIC associated proteins were identified quantified and characterized by using label free mass spectrometry. Serum albumin levels were found to be elevated in IGT, NDD and DM-MIC plasma in comparison to that of CON, which was also characterized to be AGE modified by western blotting and mass spectrometric analysis.

**Evaluation of role of glycation in autoimmune response and formation of CICs using AGE inhibitor aminoguanidine (AMG)**

To determine the role of glycation in the formation of immune complex, STZ induced diabetic mice were treated with or without prototype AGE inhibitor AMG. AMG decreased HbA1c and plasma AGEs in diabetic mice, as evidenced by fluorescence spectrometric, western blotting and mass spectrometric analysis. The annotated spectra of AGE modified peptides from mouse serum albumin and corresponding intensities were used to generate the heatmap. Mass spectrometric analysis of CICs showed elevated levels of serum albumin in diabetic mice plasma than non-diabetic mice plasma. AMG treatment reduced the albumin levels in the CICs of diabetic mice plasma. This observation was also evident by western blotting with anti-albumin antibodies. Elevated levels of albumin in CICs of diabetic mice were accompanied by a decline in plasma albumin levels. However, the decreased plasma albumin levels in diabetes were not restored by AMG treatment. In addition to serum albumin, the levels of apolipoprotein E (Apo E) (1.6), carboxylesterase 1C (1.6) and alpha 2 macroglobulin (1.4) were found to be increased in diabetic CICs. Alpha 1 antitrypsin and apolipoprotein A1 (Apo A1) were observed only in the CICs from diabetic mice plasma, but not in CICs from plasma of control mice or diabetic mice treated with AMG. These proteins were characterized to be AGE modified in mass spectrometric analysis.
Reactive immunization of mice with AGE-modified mouse serum albumin to understand immune response, its effect on glycation and albumin level.

Further we designed the experimental plan to study the effect of reactive immunization of normal mice with the CML AGE modified protein, to investigate if the immunization with self modified antigen has any effect on the level of blood glucose, glycation and AGE formation and level of serum albumin in the immune complex. The level of serum albumin in the immune complex from the control mice immunized with the CML-MSA increased significantly in comparison to the level of serum albumin in the immune complex from the control mice immunized with control-MSA. Initial results showed slight but significantly increased HbA1c in control mice immunized with AGE modified MSA accompanied by elevated serum albumin in CICs and also decrease in the total plasma albumin level.