Loss of Beta Cell Heparan Sulfate and Expression of Heparanase by Insulitis Mononuclear Cells Correlates with Type 1 Diabetes in Human Pancreas Specimens

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Purpose: Our studies have shown that heparan sulfate (HS), a glycosaminoglycan or complex sugar, is expressed strongly by mouse islets *in situ* and plays a critical role in maintaining beta cell survival. In NOD/Lt mice, T1D development correlates with progressive loss of HS in beta cells and expression of heparanase (Hpse; an endoglycosidase that degrades HS) by insulitis mononuclear cells (MNCs). Treatment of NOD/Lt mice with a Hpse inhibitor (PI-88) can prevent T1D, protect islets from destructive autoimmunity and preserve beta cell HS *in situ*. We therefore propose that beta cell HS and Hpse play critical roles in beta cell heath and T1D disease, respectively. This study investigated whether like mouse islets, normal human islets *in situ* characteristically show intense localisation of HS in beta cells. In addition, we examined whether the development of T1D in humans is associated with (i) loss of islet-associated HS, and (ii) expression of Hpse by insulitis MNCs.

Methods: HS immunohistochemistry was performed on normal (#6075, #6134, # 6096, #6094), T1D (#6052, #6062, #6069, #6045, #6145), autoantibody+ve T1D-ve (#6080, #6027, #6044, #6090) and T2D (#6108, #6109, #6059, #6028) pancreas samples. Following antigen retrieval using 0.05% pronase, sections were stained using mouse 10E4 mAb (Seikagaku) that recognises highly sulfated HS, horseradish peroxidase (HRP)-conjugated rabbit anti-mouse Ig and 0.04% 3-amino-9-ethylcarbazole (AEC)/0.015% hydrogen peroxide. Mouse IgM was used as the isotype control. Quantitative analyses of the intra-islet area of 10E4+ve staining was done using Image J software with color deconvolution plug-in (n=4/group for normal, T1D or T2D pancreas specimens, 10 islets examined/specimen). For antigen retrieval and Hpse immunohistochemistry, sections were initially heated in citrate buffer (pH 6.0) and then stained using HP130 mouse anti-Hpse mAb (Insight), the PK-2200 M.O.M. Immunodetection kit (Vector Labs) and AEC/0.15% hydrogen peroxide. Mouse IgG1 was used as the isotype control. For insulin and glucagon immunostaining, mouse anti-insulin and mouse anti-glucagon mAbs (Sigma) were used with the M.O.M. kit.

Summary of Results: HS was strongly expressed in normal human islets *in situ* and correlated with the immunolocalisation of insulin+ve beta cells and not glucagon+ve alpha cells, suggesting that the HS was localised in islet beta cells. Intense HS staining was similarly found in islets of autoantibody+ve T1D-ve pancreas samples. In contrast, islets from T1D specimens, showed low or no intra-islet staining for HS even in the presence of strong insulin staining (#6052 and #6069). Quantitative analyses revealed a 10-fold reduction in the HS content of T1D islets compared to normal islets ($P<0.0001$) and a 3-fold reduction in T2D islets ($P<0.0001$). Staining for Hpse was found to be intense on insulitis MNCs and prominent in some islets in T1D specimen #6052 (at 1 year post-onset). In contrast, Hpse staining was variable in normal human islets with weak intracellular or cell surface localisation.

Conclusions: These findings indicate that progression of T1D disease in humans correlates with loss of beta cell HS, probably due to the production of Hpse by insulitis MNCs and possibly by the beta cells. Loss of HS in islet beta cells may represent a common pathway leading to the decline in beta cell viability for both T1D and T2D.