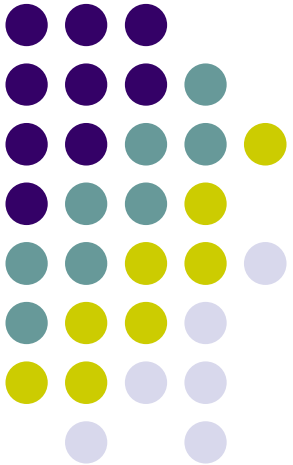
Linköpings universitet

Identification and characterization of the IC2 auto antigen on the pancreatic beta-cells

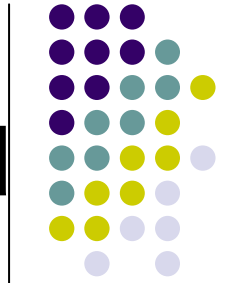
Master thesis presented by- Md. Golam Kafi Afrose Mia



Supervisor: Carl Henrik Brogren,

Copenhagen University, Denmark

Diabetes Melitas; a thread to mankind



- Diabetes; A Leading Cause of Death

- Diabetes currently affects 246 million people worldwide and is expected to affect 380 million by 2025.

- The economic and social costs of diabetes are enormous.

- In developed countries, 10% or more of the total health budget is spent on the management of diabetes and its complications.

Jonsson, 2002; Zimmet et al, 2003 Eurodiab Study Group 2001.
International Diabetes Federation.

Diabetes-

metabolic disorder characterized by beta cell loss and insulin deficiency.

blood glucose level rises abnormally.

treatable but it is still incurable.

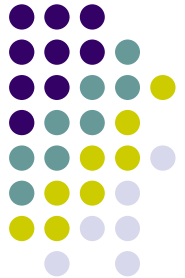
Type-1 Diabetes (TID)-

The pancreas is unable to produce insulin.

Type-2 Diabetes-

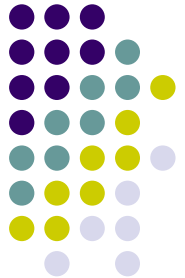
Pancreas can produce insulin but it is unable to metabolise blood glucose.

IC2?



- Beta-cell surface specific monoclonal autoantibody and stable hybridoma.
- Derived from LPS–stimulated spleen cell of a non-immunised diabetic BB-rat.
- Fulfilled both the specificity and affinity criteria required for in vivo imaging of native beta cells.

Why IC2 autoantigen?



- May be it involves in pathogenesis of type-1 diabetes.
- So, Identification and characterization of this autoantigen is important to understand the pathogenesis of TID.

Proposed Molecular Mechanism of IC2

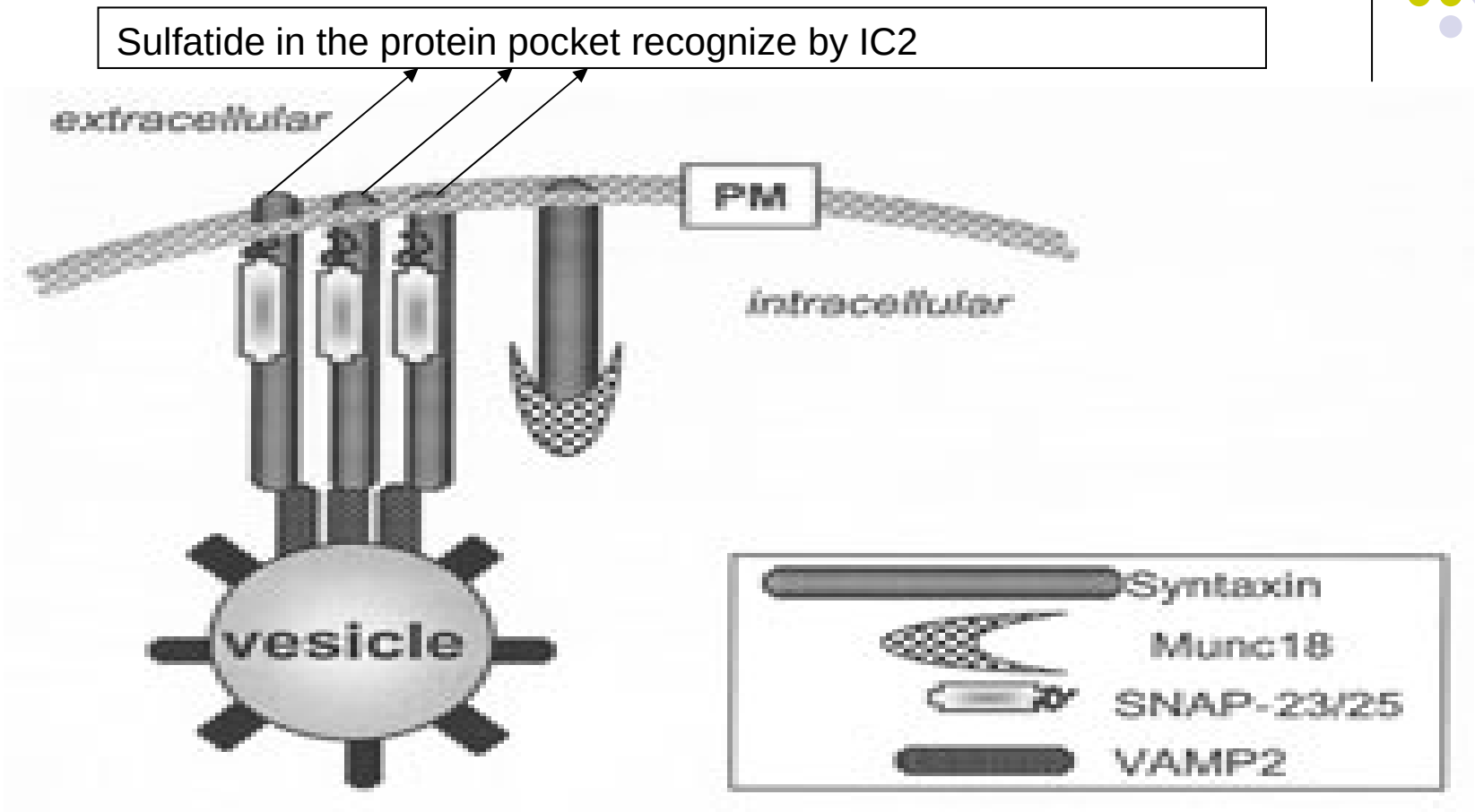


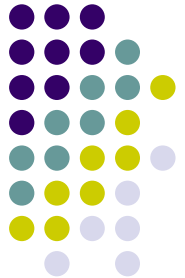
Figure- SNARE proteins and their function in insulin secretion also dis-regulated by Sulfatide

Hypothesis

We hypothesise that Sulfatide on the pancreatic beta cell plasma-membrane is the target epitope of IC2.

To re-establish the findings of Professor Spitalnik, *Columbia University* (1991).

Materials and Methods



Cell Lines

•β cells: Rat pancreas cell (AR42J) and Rat insulinoma cell (RIN-5AH).

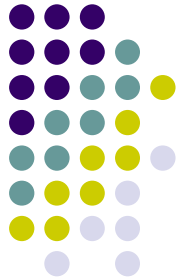
•α cell: Pancreatic T cell (α TC19).

•Rat mucosal mast cell (RBL cell).

•IC2, hybridoma cells derived from Rat myeloma Y3.

Materials and Methods

Contd.....



TLC plates / Stationary phase

Silica gel coated both AL2OH (MERCK, Cat no-1055540001)

Glass plates (MERCK, Cat no-1137480001)

Mobile phase

Chloroform: Methanol: Water (5:4:1, 3:4:1, 8:4:1 6:8:1, 1:5:1, 5:1:1, 2:5:1, 1:10:2)

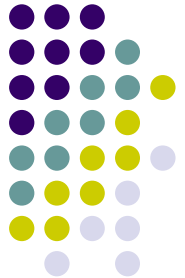
2-Methyl propanol: Ethanol: H2O: 28% NH4OH (100:67:67:2.3) were used

Different chemical staining

Primulin, Orcinol, Thymol, Carbazole, Cu-acetate and Fluorescence

Materials and Methods

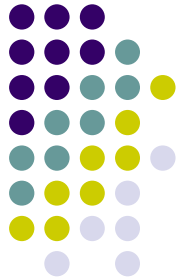
Contd.....



- Cell expansion up to NUNC cell factory.
- Plasma membrane isolation by applying “French press technique” and more pure lipids were extracted from plasma membrane.
- Lipid extraction (Folch Method).
  - From cells
  - From plasmamebrane

Materials and Methods

Contd.....



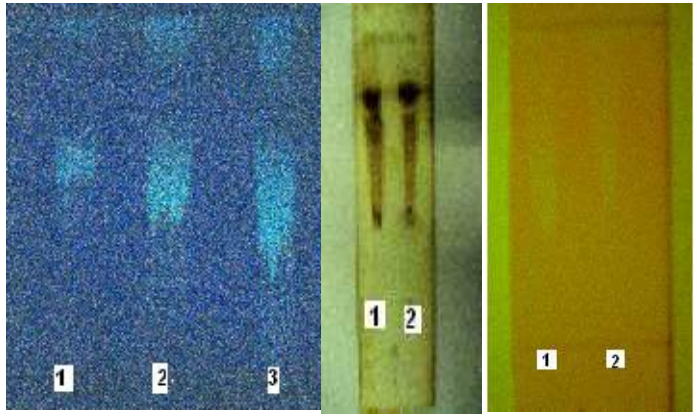
- TLC & HPTLC experiments on these lipids.

- Different chemical staining methods (Primulin, Orcinol, Carbasol, Cu- acetate and Thymol spray) to detect lipid separation bands from both TLC and HPTLC plates.

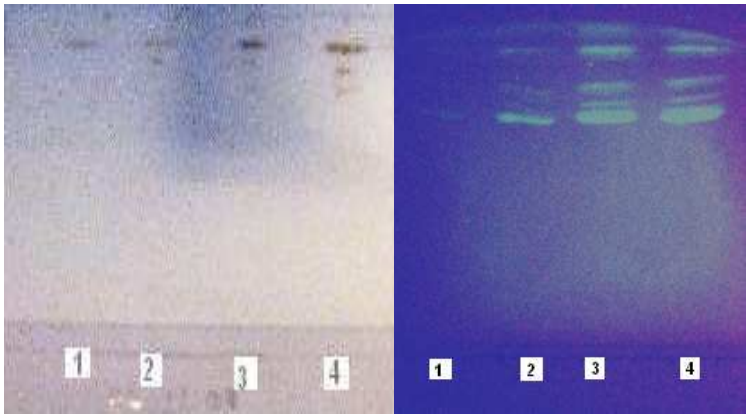
- Immuno blotting of TLC and HPTLC plates (plan to perform on the June-July,2009).

Results & Discussion

Lipid separation bands from TLC (left) and HPTLC (right) experiments



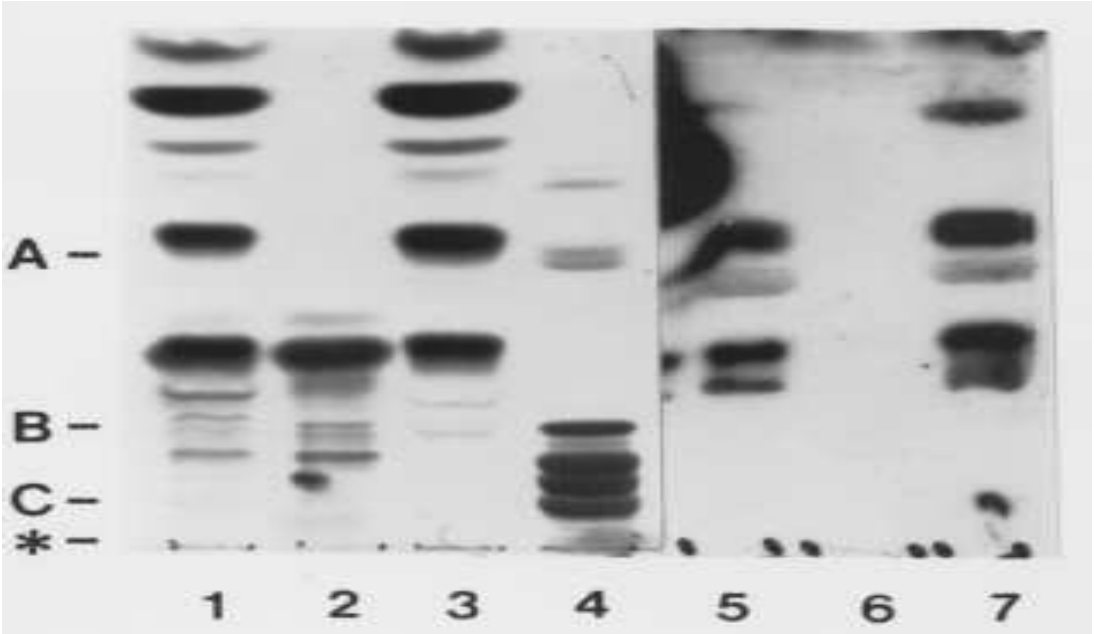
TLC experiments of RIN 5AH lipids staining with Cu-acetate (left), Carbasol (middle) and 2/7- dichlorofluroscence (right) methods.



HPTLC experiments on RIN 5AH(left) lipids staining with orcinol spray and IC2 (right) lipids staining with primulin spray.

Results & Discussions Contd.....

Figure by Spitalnik et al,1991



•RINm5F glycolipids were isolated, seperated by TLC. Bands are shown by applying orcinol staining (lane 1-4) and immunostaining by IC2 (lane 5-7). Here, the position of human Brain cromatographic Standards galactosyl sulfatide, ganglioside GM-1b ganglioside GT-1b are indicate by A-C respectively. (*) denotes the origin. Lane 1 & 5 RINm5F total lipid extract. Lane 2 & 6 RINm5F Folch upper phases lipids. Lane 3&7 RINm5F Folch lower phases lipid. Lane 4 human Brain glycolipid. (Fig-1 Spitalnik et al, 1991)

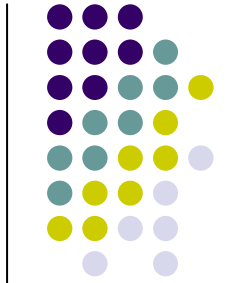
Conclusion

- We have got better lipid separation in HPTLC techniques than TLC.

- Chloroform: Methanol: 0.25% aqueous KCL 5:4:1 was the best mobile phase to get better lipid separation bands.

- Nice bands were detected by primulin spray (Fig 6,8,10 & 11) but orcinol spray also effective.

Future steps



- To re-establish the hypothesis that IC2 binds with the auto antigen on the pancreatic beta-cell plasma membrane, we are just far from the last immunobloting step.

- Both cell lipids and plasmamembrane lipids should be used to get more clear knowledge.

- Different types of (both alfa and beta)cells should be applied to get more comparaable result.

Thanks for your patience