Inhibition of the Zonulin-dependent increased Intestinal permeability can prevent the onset of type 1 diabetes in BB/Wor.

BACKGROUND

Several studies suggest that an increased intestinal permeability (IP) due to alteration of intestinal tight junction (TJ)can be involved in the pathogenesis of many autoimmune diseases,including type 1 diabetes.

BACKGROUND

We have recently described a novel protein, zonulin, that dictates TJ competency. Structure-function analysis of the protein allowed us to engineer a synthetic peptide (FZI/0) that prevents zonulin binding to its target receptor.

AIM

1.To establish the role of zonulin-dependent intestinal permeability in the pathogenesis of Type 1 diabetes.

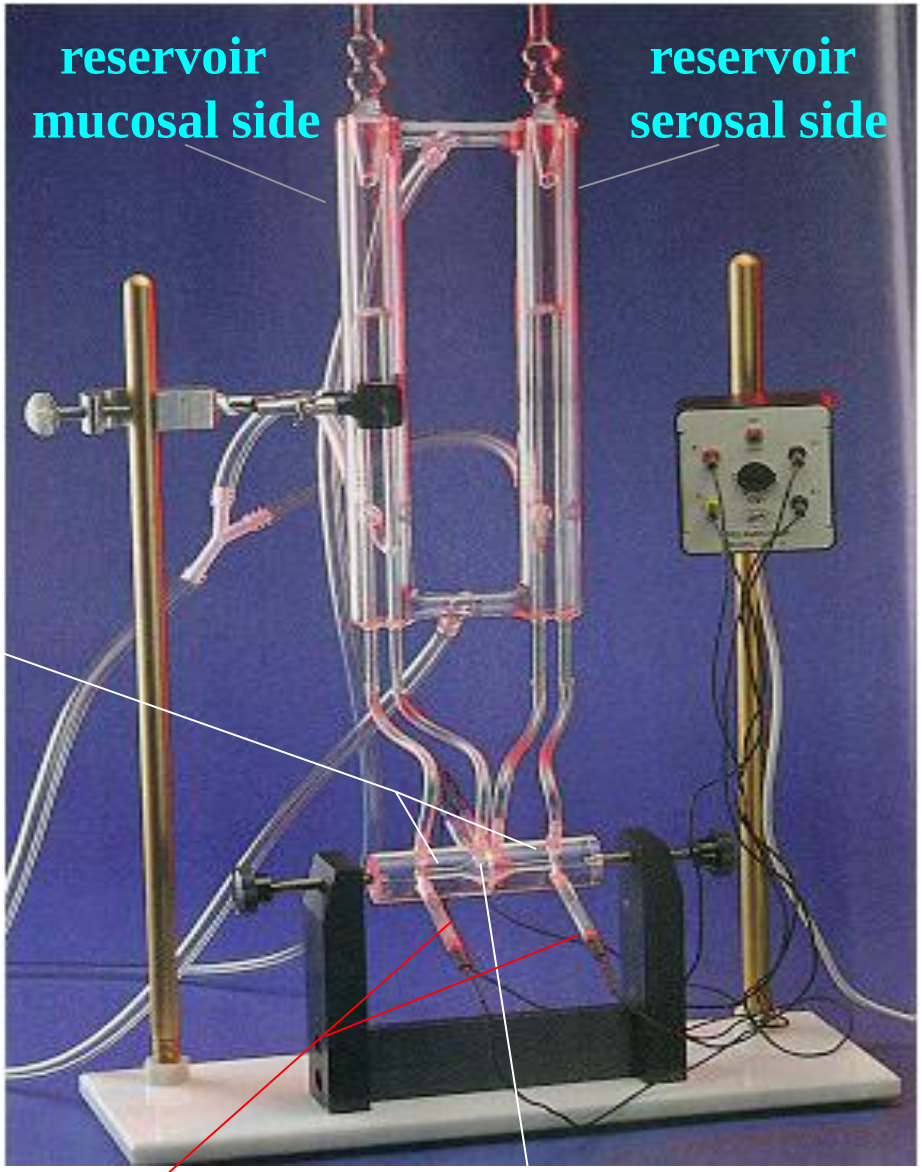
2.To establish whether zonulin-inhibition can prevent the onset of Type 1 diabetes by affecting intestinal permeability.

METHODS

Ex-vivo studies:

Small intestine of both BB/Wor diabetic prone (DP) and diabetic resistant (DR) rats were mounted in Ussing Chambers to measure trans epithelial intestinal resistance (TEER). Measurement of intraluminal zonulin was determined by sandwich ELISA.

Ussing Chamber



METHODS

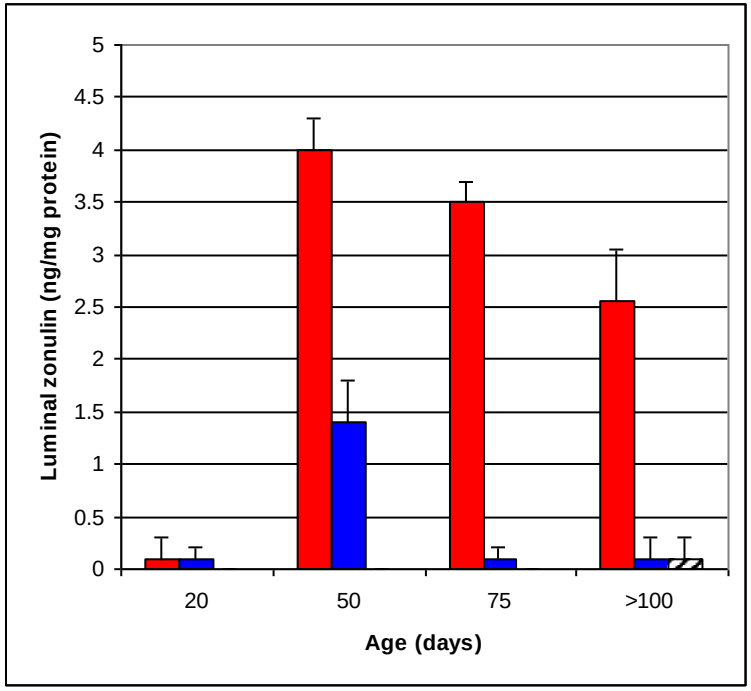
In vivo studies:

BB/Wor rats were randomized at age 20 days into a treatment group (n=15) that received drinking water plus 10 μg/ml FZI/0 in bicarbonate, and a placebo group (n=15) that received only bicarbonate. In vivo IP using Lactulose/Mannitol (LA/MA) assay and serum zonulin and glucose levels were monitored every 10 days. Rats with blood glucose>200mg/dl were considered diabetic and were sacrificed within 24 hours of reaching the diabetic status.

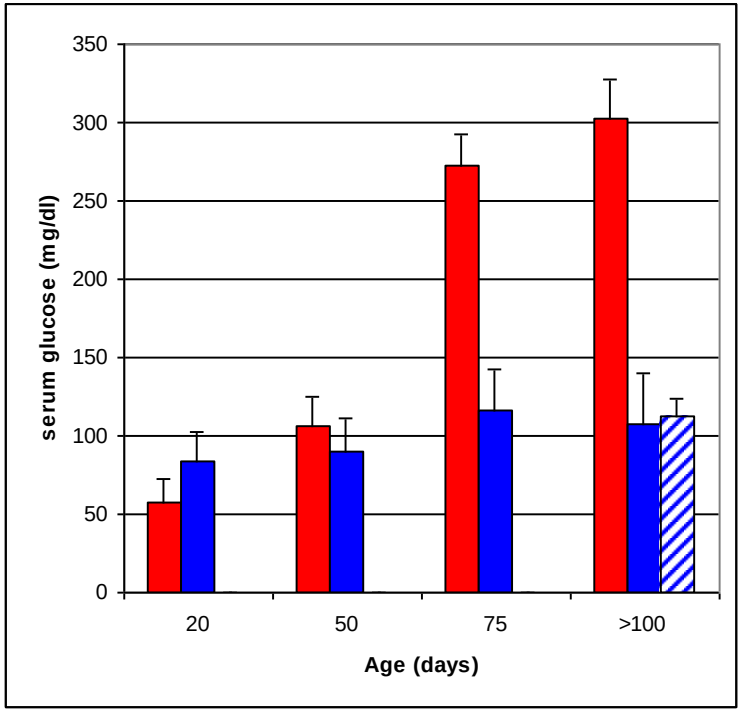
RESULTS

Ex-vivo studies

A



B

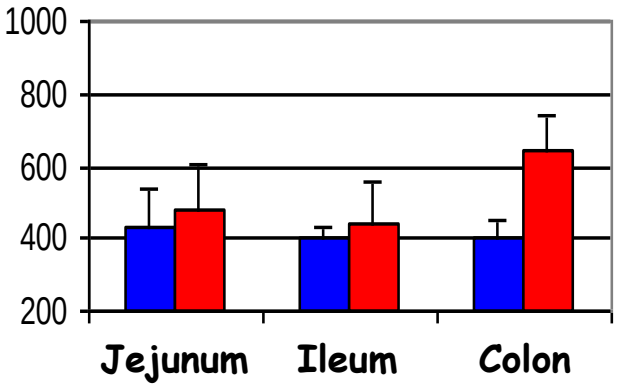


Intraluminal levels of zonulin (A) and serum glucose levels (B) in both BB/Wor DP (red bars) and DR rats (blue bars) at increasing ages. The DP rats that did not progress to Type I diabetes (~15%) (dashed bar) had low levels of intraluminal zonulin and normal glucose levels. Average age of onset of diabetes in DP rats was 69.2±2.9 days. N=3-6 for each group

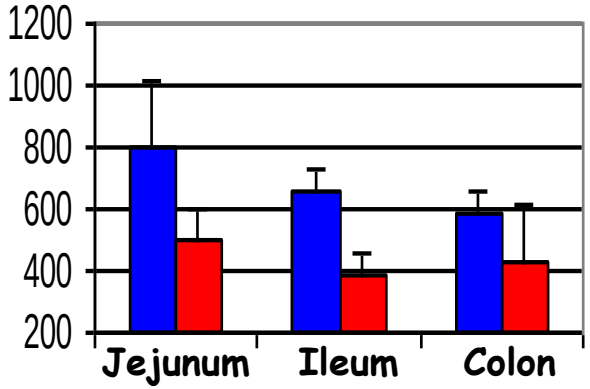
RESULTS

Ex-vivo studies

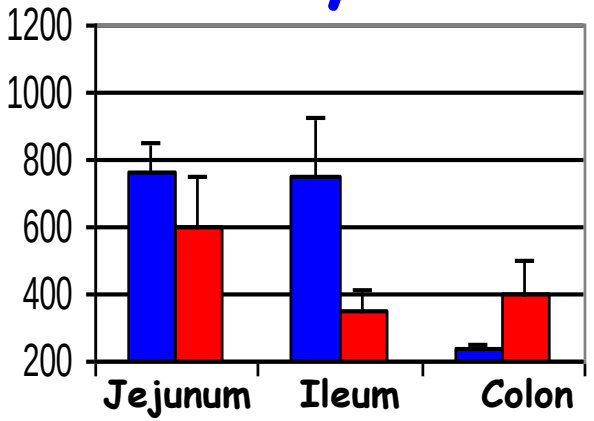
20 days old



50 days old



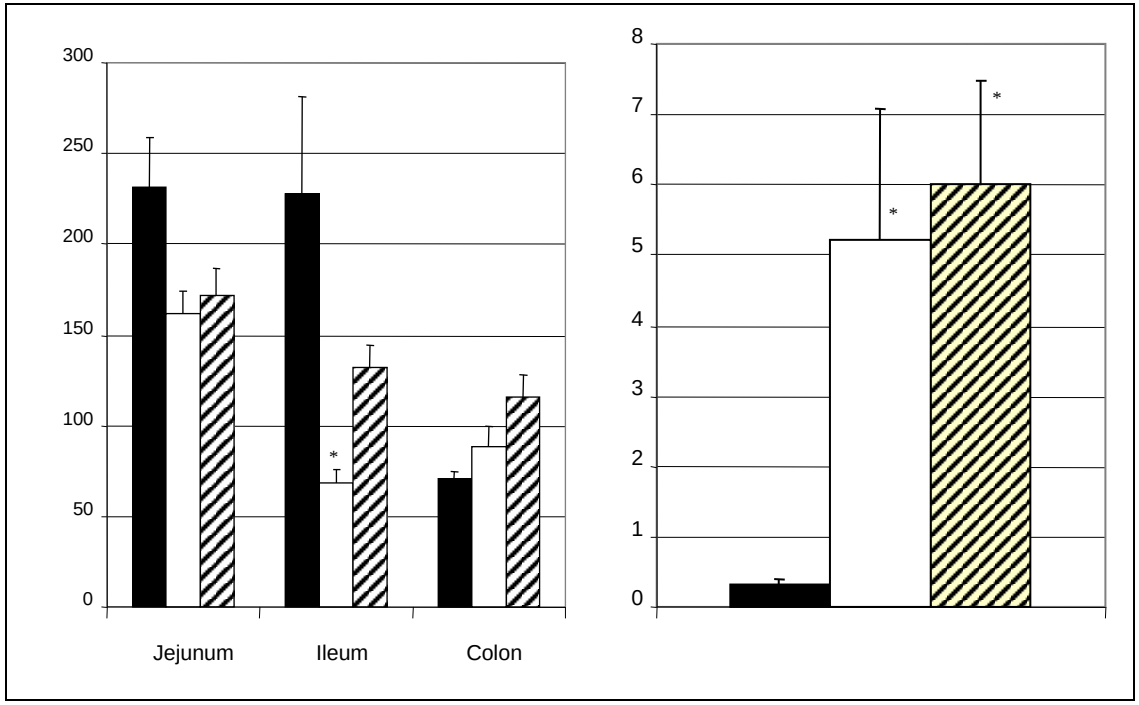
75 days old



Intestinal resistance (TEER, Ω.cm2) in DP (red bars) and DR (blue bars) BB/Wor rats. No difference in TEER between DR and DP rats were observed at age 20 days, irrespective of the intestinal tract examined (left panel). By age 50 days, the TEER of the small intestine in DP animals was significantly lower both the jejunum and ileum, while the colon showed no differences in TEER between the two groups (central panel). Similar differences in TEER in the ileum were observed also at age 75 days (right panel). N=7 for each group.

RESULTS

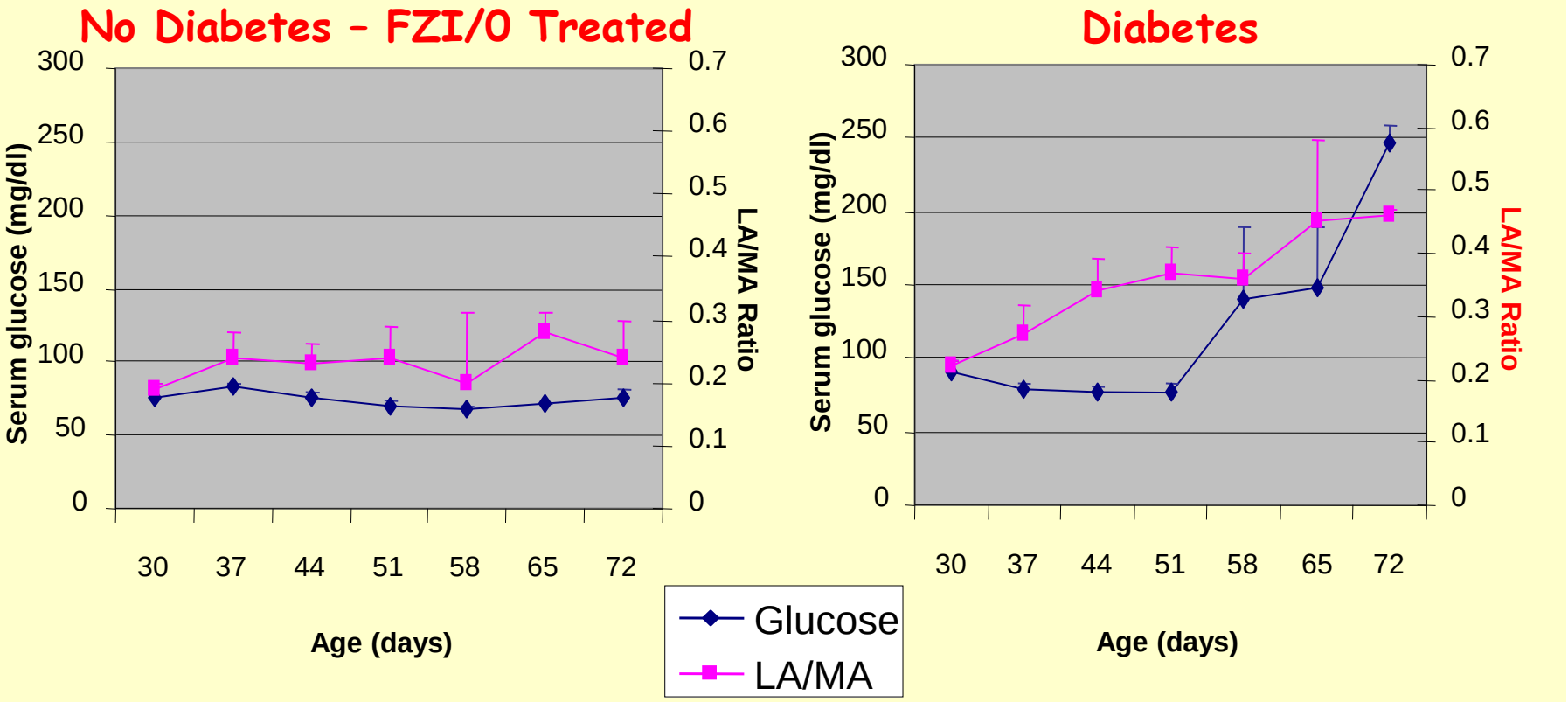
Ex-vivo studies



Effect of the zonulin inhibitor FZI/0 on TEER (Ω.cm2, left panel) and zonulin release (ng/mg protein, right panel) in BB/Wor DP rats. Untreated DP rats (open bars) showed a significant decrease in ileal TEER and increase in luminal zonulin compared to DR animals (closed bars). FZI/0 treatment (dashed bars) prevented the ileal TEER decrement without affecting the intraluminal zonulin release. N=7-15 for each group. *$p<0.02$.

RESULTS

In-vivo studies

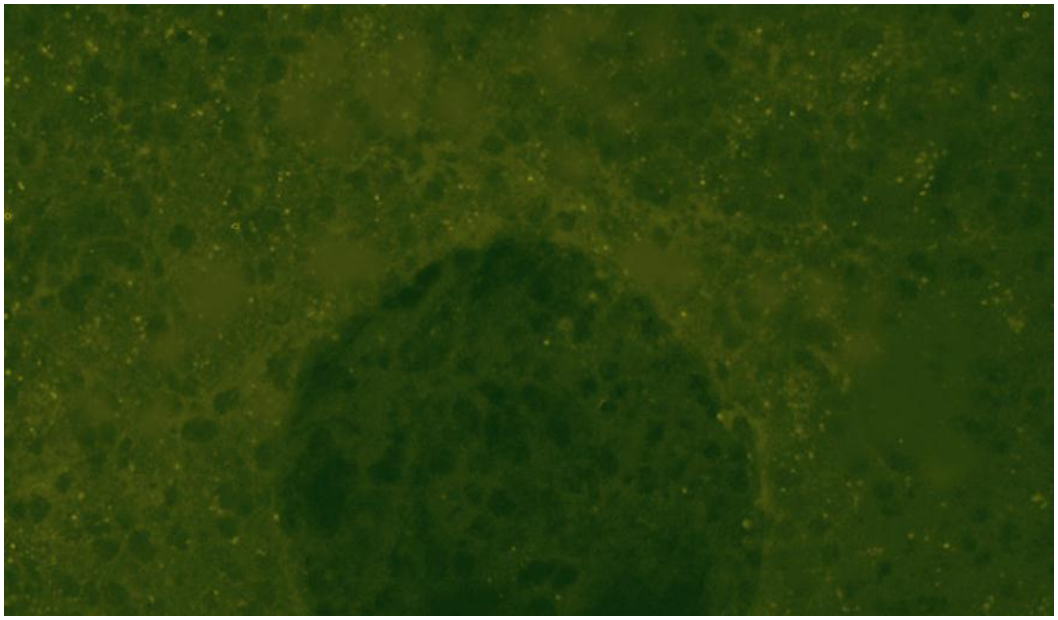


In vivo intestinal permeability and serum glucose in DP rats. DP rats treated with FZI/0 and that did not develop diabetes (A) had no changes in intestinal permeability as established by LA/MA ratio (squares). Conversely, untreated DP animals that evolved to diabetes (B) showed an increase in intestinal permeability that became statistically significant at age 44 days. No significant changes in serum glucose levels (diamonds) were observed in FZI/0-treated rats (A), while in untreated animals there was a significant increase in glicemia starting approximately two weeks after the increase in intestinal permeability (B). N= 15 for each group

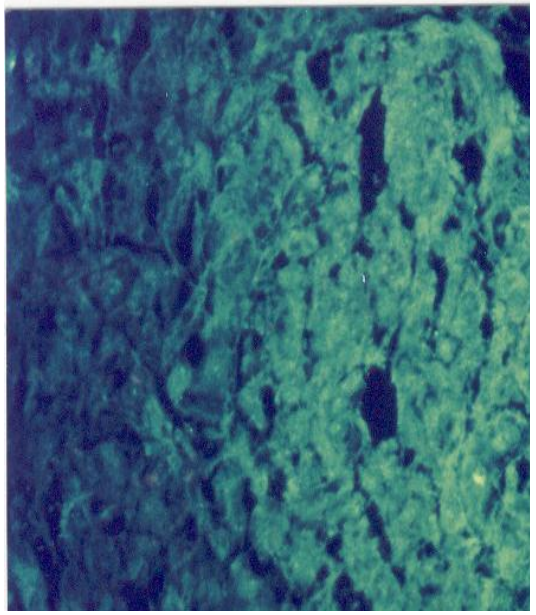
RESULTS

In-vivo studies

ICA Negative



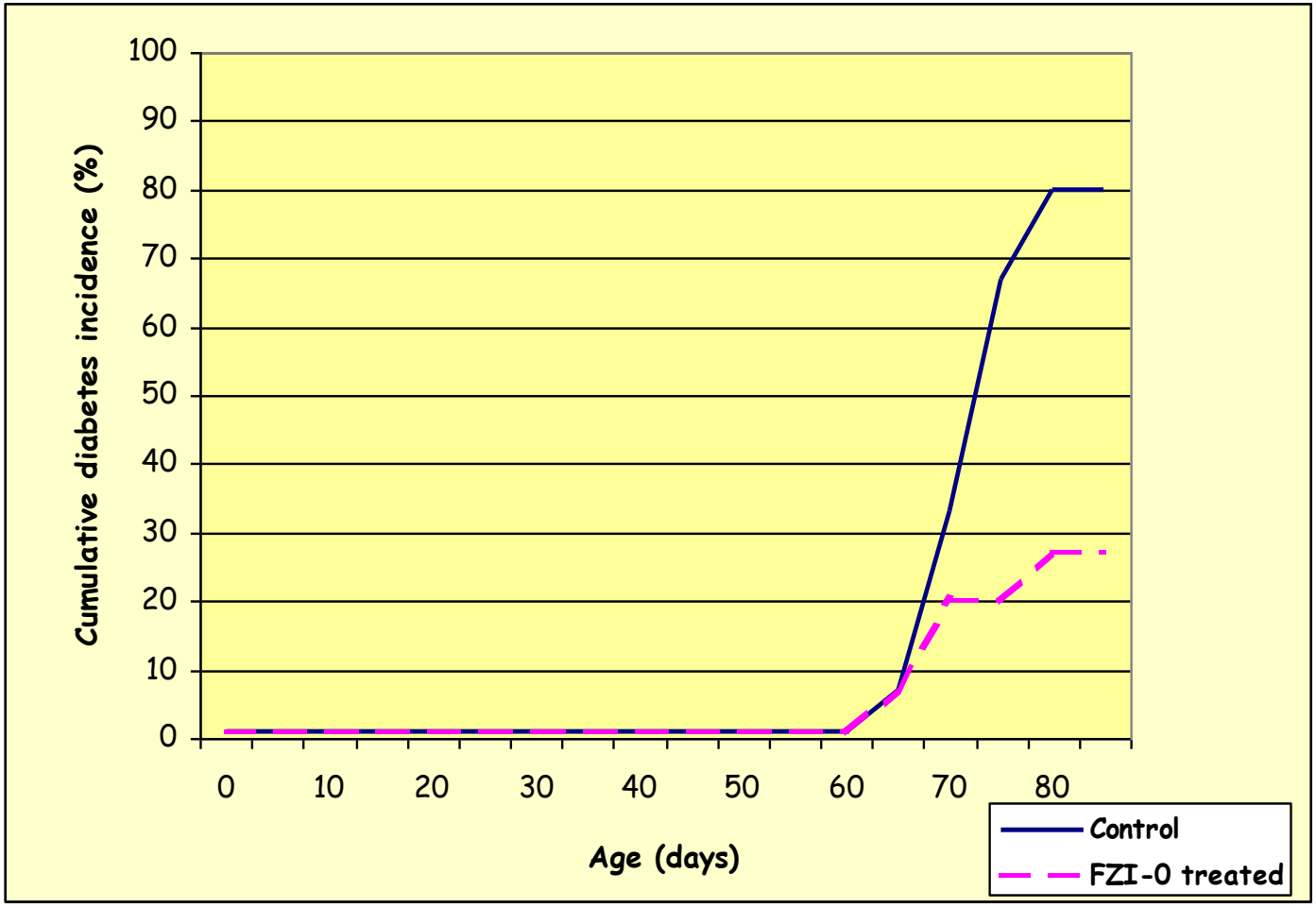
ICA Po



Negative islet cell antibodies (ICA) in FZI/0 treated rats that did not develop diabetes (left panel) compared to ICA positive in untreated diabetic animals.

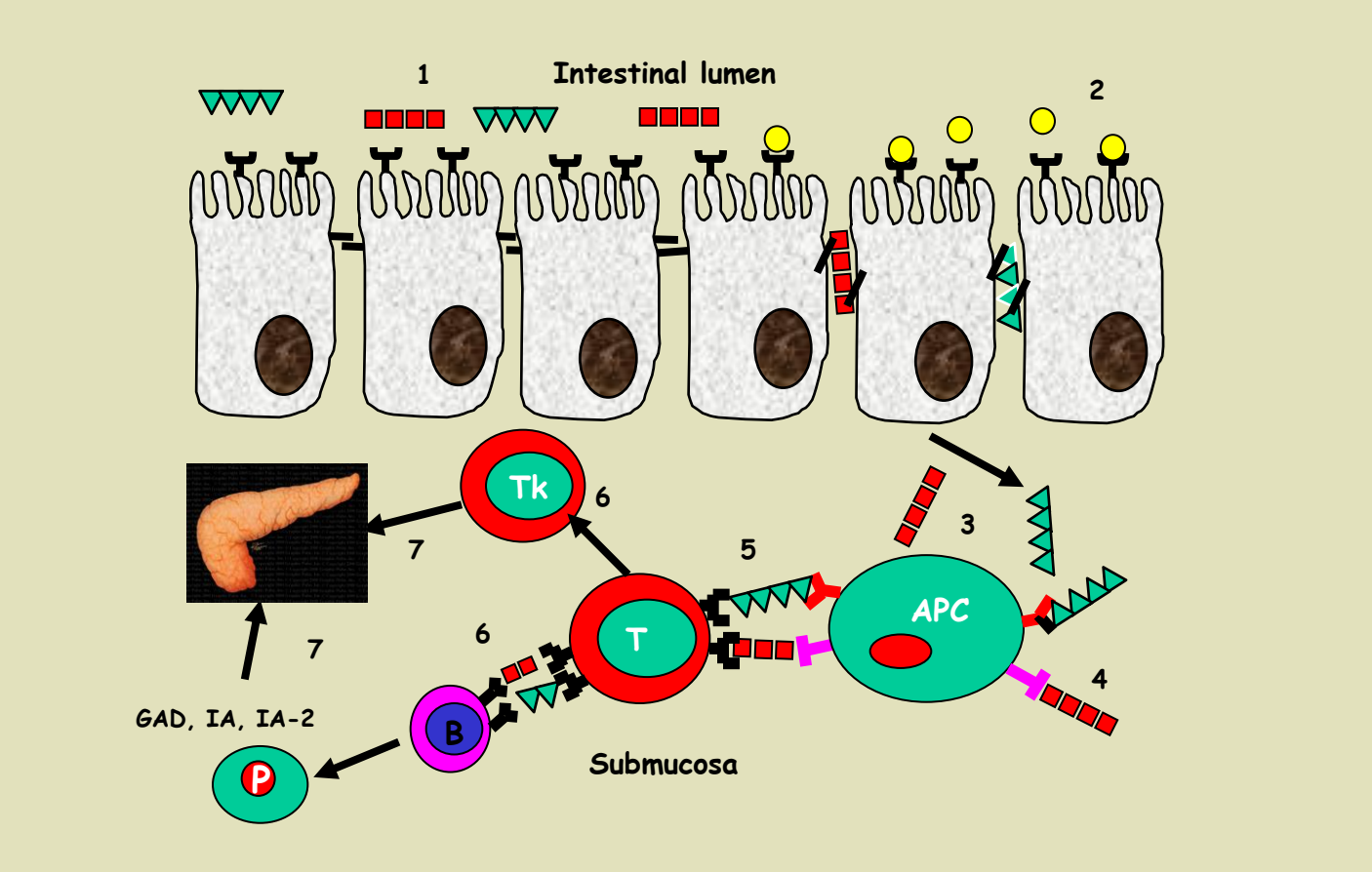
RESULTS

In-vivo studies



Cumulative incidence of type 1 diabetes in BB Wor DP rats treated with either buffer control (continuous line) or with the zonulin inhibitor FZI/0 (dashed line). N=15 for each group

PROPOSED MODEL



Proposed role of aberrant intestinal permeability in Type 1 diabetes pathogenesis. Non-self antigens are present in the intestinal lumen (1) and cross the tj barriers in subjects with dysregulation of the zonulin system (2-3). Antigen peptides bind to HLA receptors present on the surface of APC (4). In turn, these peptides are presented to T lymphocytes (5). In genetically susceptible individuals, an aberrant immune response (both umoral and cell-mediated) (6) leads to the autoimmune process mainly targeting the Langherans islets with subsequent insulin deficiency typical of type 1 diabetes (7).

CONCLUSIONS

- There is a correlation between intraluminal zonulin and IP in BB/Wor DP but not DR animals.
- The increased IP precedes the onset of diabetes.
- Daily administration of the zonulin binding inhibitor FZI/0 blocks the zonulin permeating effect in the gut, so preventing the onset of Type 1 diabetes in genetically susceptible BB/Wor rat.