

Inhibition of the zonulin pathway blocks the progression from pre-clinical autoimmunity to Type 1 diabetes in BB/wor rats.

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Inhibition of the zonulin pathway blocks the progression from pre-clinical autoimmunity to Type 1 diabetes in BB/wor rats.

Background: We have previously studied that zonulin, a protein involved in tight junctions modulation, is up regulated in BB/wor rats and is responsible for the increased intestinal permeability (IP) typical of this animal model of autoimmunity. We have also demonstrated that by blocking the zonulin pathway before the onset of autoimmunity, the incidence of Type 1 Diabetes (T1D) can be prevented in 75% of the animals.

Aim : To determine whether the progression of T1D can be blocked, even though the autoimmune process is already established .**Methods:** 30 BB/ Wor rats were randomized after immune seroconversion (average age 55 days) in a treatment group (N=20) that received autoclaved water supplemented with 3mg /ml of zonulin receptor blocker AT1001 and HCO_3^- 1.5g/100ml to buffer gastric acidity, and a placebo group (N=10) that received only water with HCO_3^- . Serum zonulin and autoantibody levels were monitored at the beginning of the study and at its endpoint. Water intake was monitored daily, weight gain and serum glucose levels were checked weekly. Rats with fasting blood glucose ≥ 250 mg/dl were considered diabetic and were sacrificed within 24 hours of reaching the diabetic status.

Results: Six out of 10 (60%) untreated rats developed T1D, while only 7/20 (35%) of the AT1001-treated animal progressed to T1D. The average age of onset of T1D was 85.4 ± 10.4 in the placebo group and 86.0 ± 10.3 in the treated group. AT1001 treatment did not change the serum zonulin levels between beginning (average age 55 days) and the endpoint of the experiment (average age 100 days) of the experiments. Conversely, the anti-glutamic acid decarboxylase (GAD) antibodies were significantly reduced in AT1001-treated rats that did not develop T1D (0.87 ± 0.35) compared to the treated animals with the disease developed (1.87 ± 0.59 ; $p < 0.05$)

Conclusions: The blockage of the zonulin pathway in BB/wor rats at their preclinical autoimmune stage significantly reduced the progression to T1D. This decreased incidence of T1D was associated to a significant reduction of the anti-GAD antibodies following AT1001 treatment.

Background

We have previously demonstrated that zonulin, a protein involved in tight junctions modulation, is up regulated in BB/wor rats and is responsible for the increased intestinal permeability (IP) typical of this animal model of autoimmunity.

We have also showed that by blocking the zonulin pathway before the onset of autoimmunity, the incidence of Type 1 Diabetes (T1D) can be prevented in 75% of the animals.

Aim

To determine whether the inhibition of the zonulin pathway can block the progression of T1D in BB/Wor rats, even if the autoimmune process is already established .

Methods

Treatment of DP rats with zonulin inhibitor AT-1001 ("FZI/0")

- BBDP animals age 52-54 days divided in two groups
- Group 1 (n=20): AT-1001 daily in drinking water + HCO₃
- Group 2 (n=10): drinking water + HCO₃

Methods

Serum zonulin and autoantibody levels were monitored at the beginning of the study and at its endpoint. Water intake was monitored daily, while weight gain and serum glucose levels were checked weekly. Rats with fasting blood glucose ≥ 250 mg/dl were considered diabetic and were sacrificed within 24 hours of reaching the diabetic status.

BB/wor DP Treatment Study: Study Design

Age: 52-54 days

Steps

Baseline

AT1001 Treatment

T₁

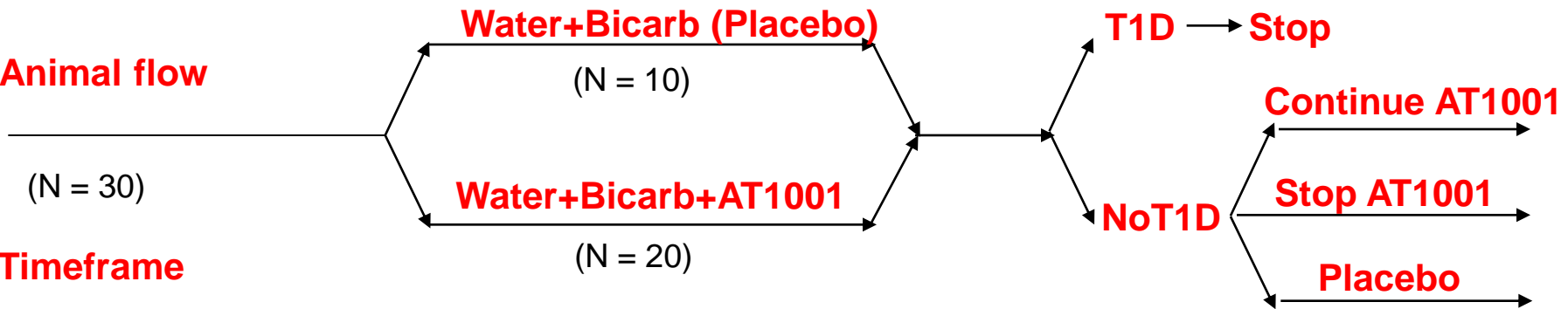
Intervention

Weight
Serum Glucose
ICA
Daily water intake

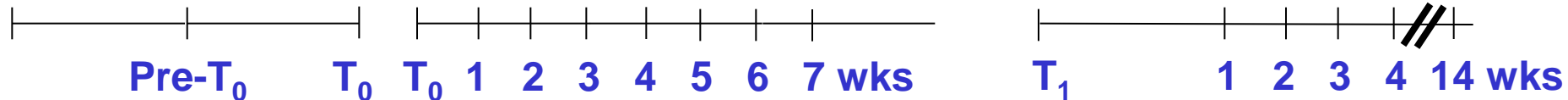
Randomization
Water intake (daily)
Weight (weekly)
Serum glucose (weekly)

Serum glucose
ICA

Animal flow



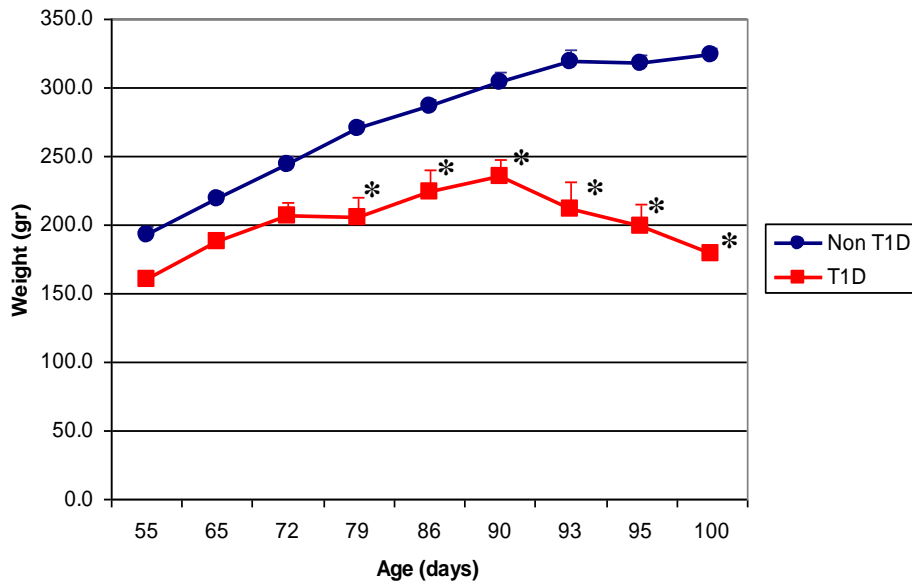
Timeframe



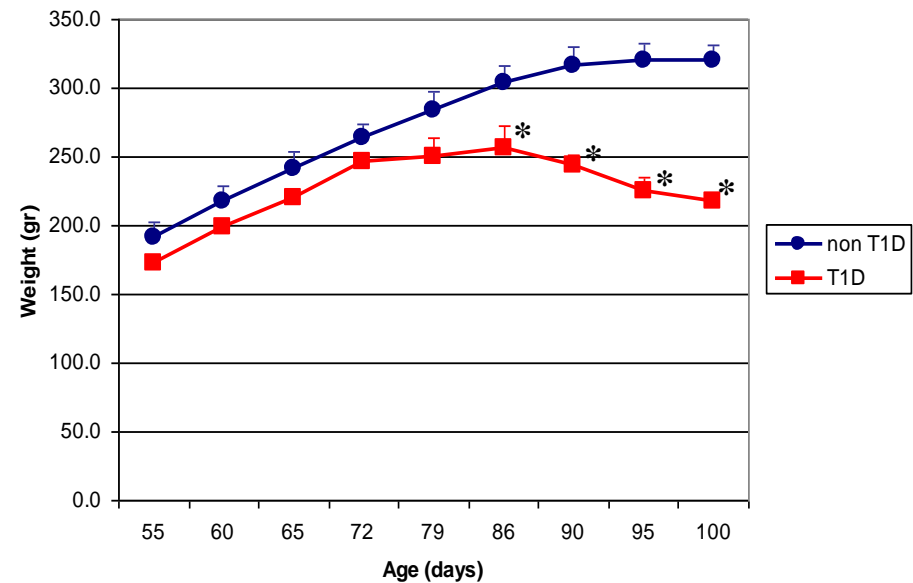
RESULTS

BB/wor DP Treatment Study: Weight Gain

AT1001 treated group



Placebo Group

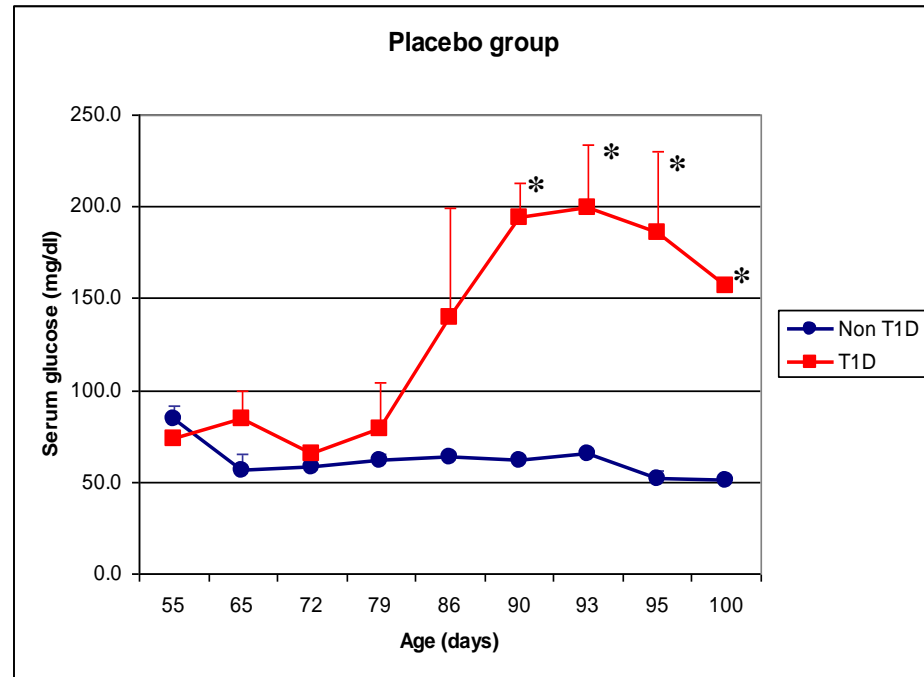
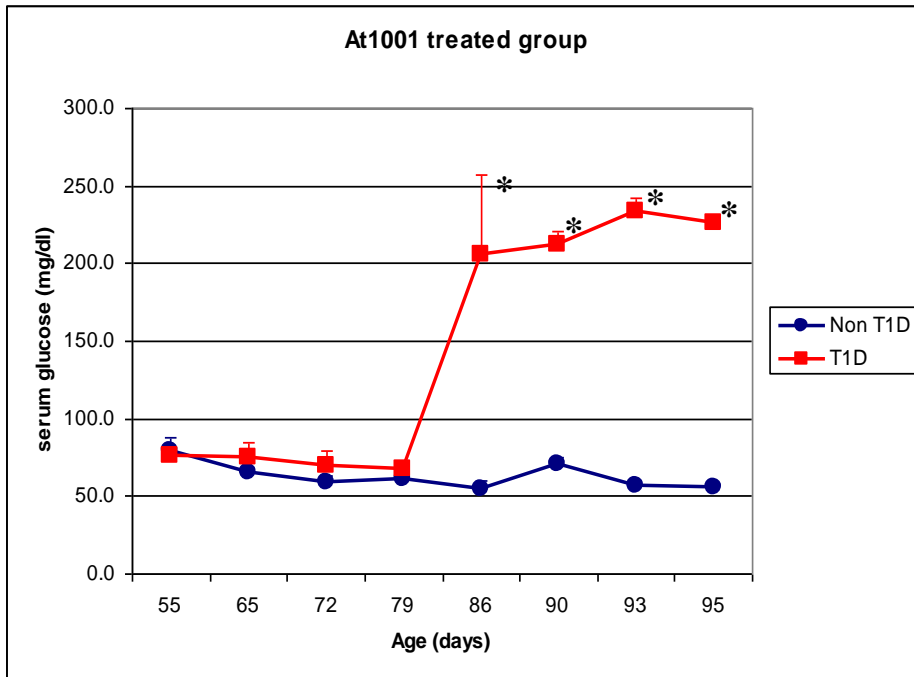


*p<0.01 compared to non-T1D

BB/wor rats that developed T1D showed a lower weight gain starting at age 79-86 days compared to rats that did not develop the disease, irrespective of the treatment regimen.

RESULTS

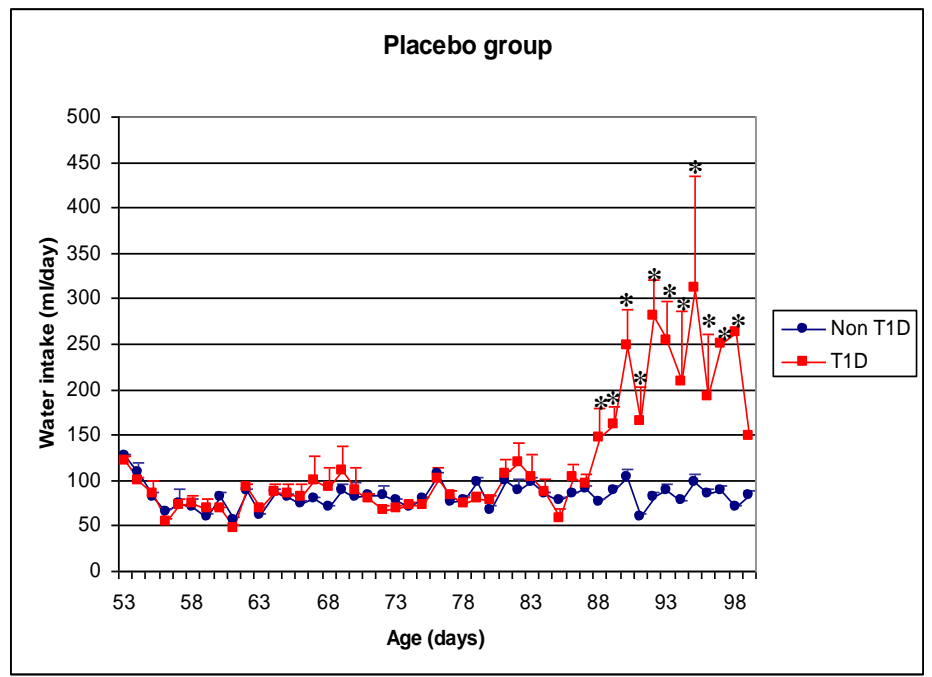
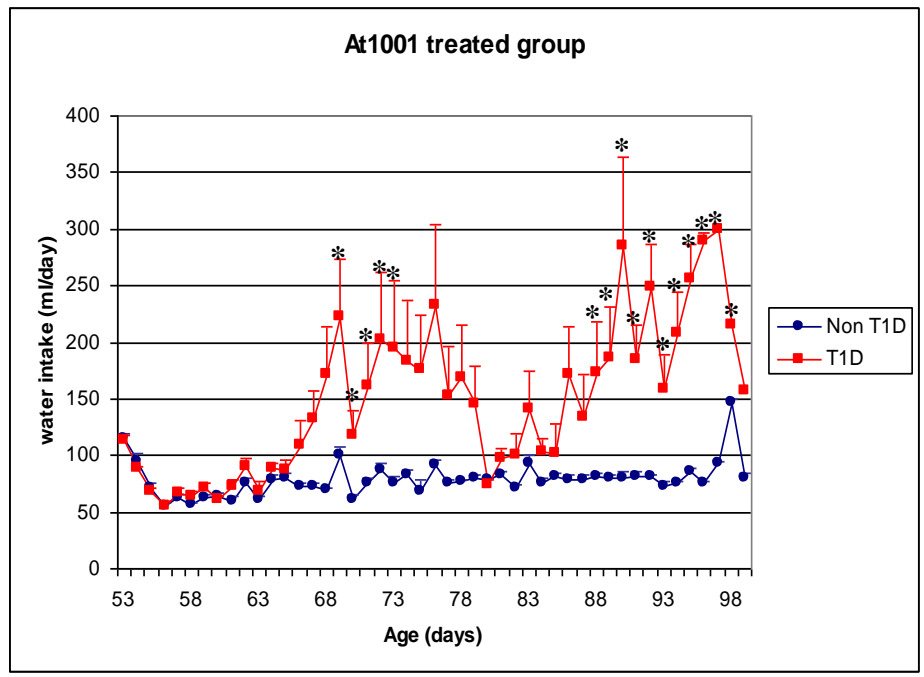
BB/wor DP Treatment Study: Serum Glucose Levels



*p<0.01 compared to non-T1D

RESULTS

BB/wor DP Treatment Study: Water Intake



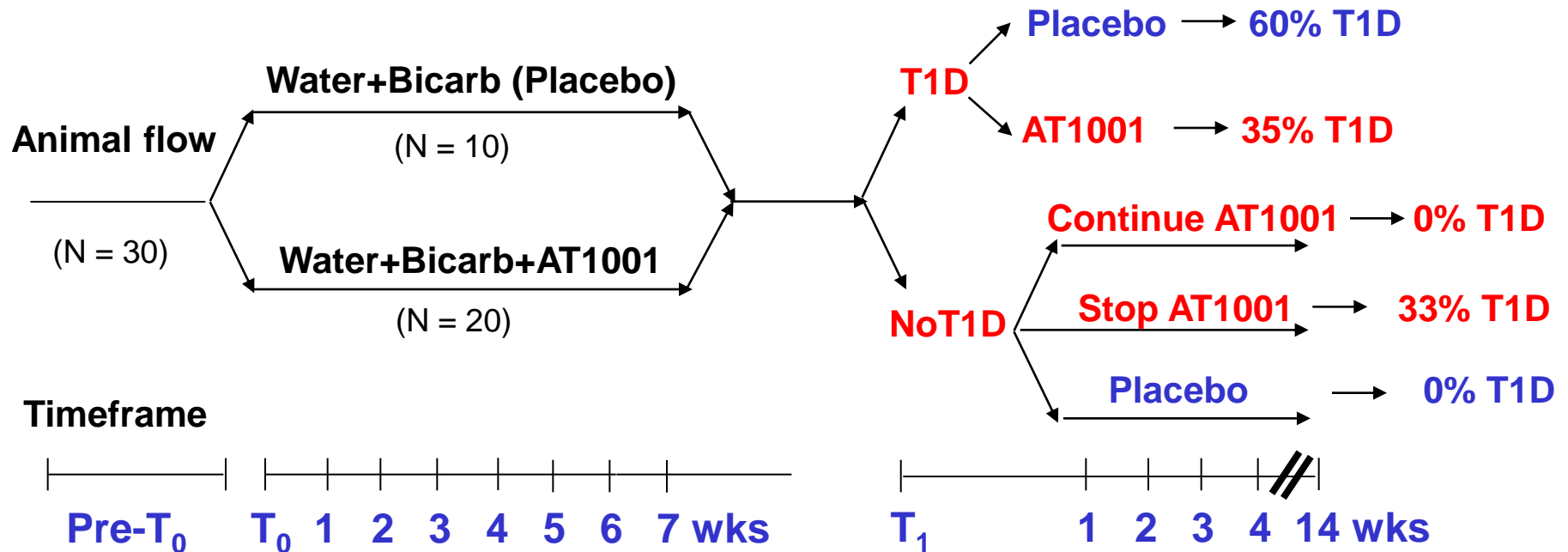
*p<0.05 compared to non-T1D

BB/wor DP Treatment Study: Results

Age: 52-54 days

Steps

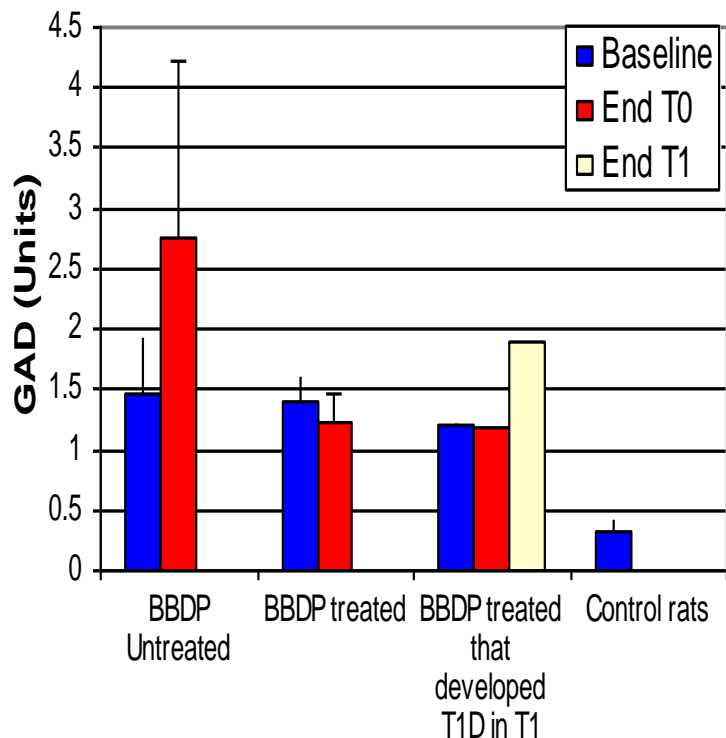
Baseline **AT1001 Treatment**



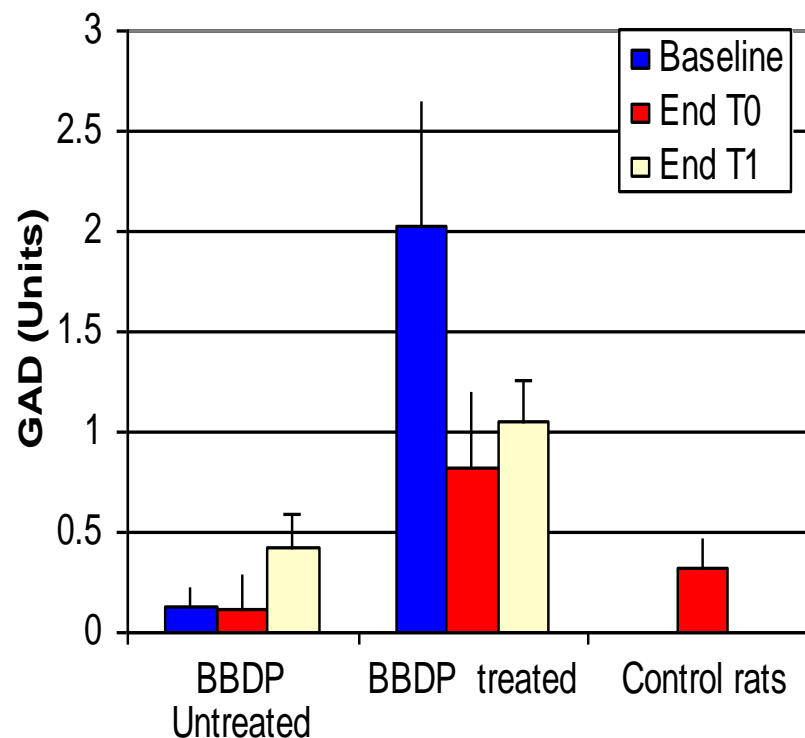
Diabetes Treatment Study: Treatment of Autoimmune Diabetes with AT-1001

Changes in auto-antibodies

BB/Wor rats that developed T1D



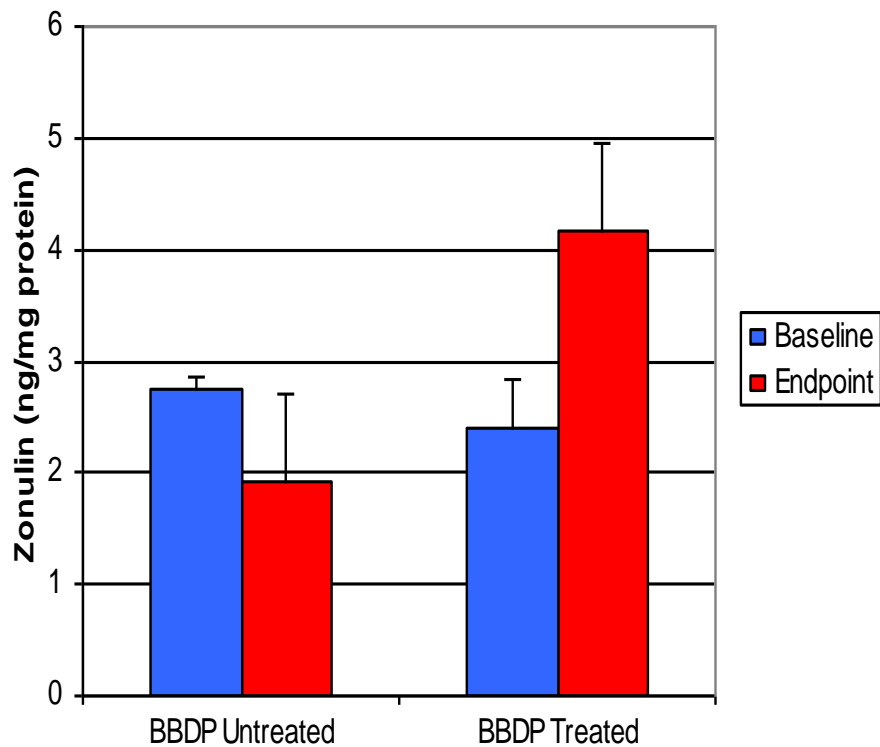
BB/Wor rats that did not develop T1D



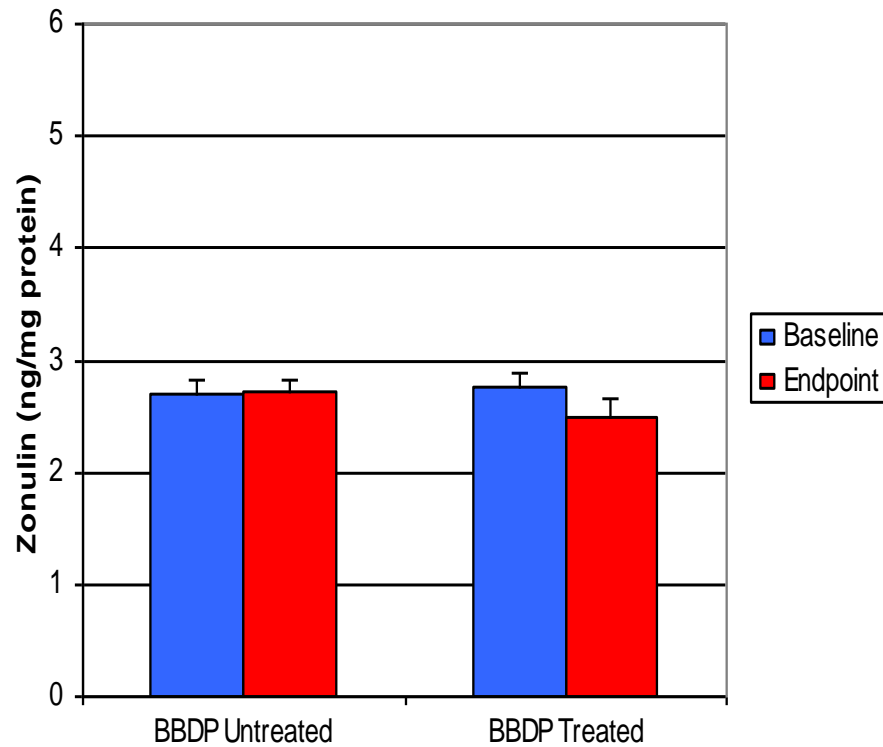
Diabetes Treatment Study: Treatment of Autoimmune Diabetes with AT-1001

Changes in Serum Zonulin

BB/Wor rats that developed T1D



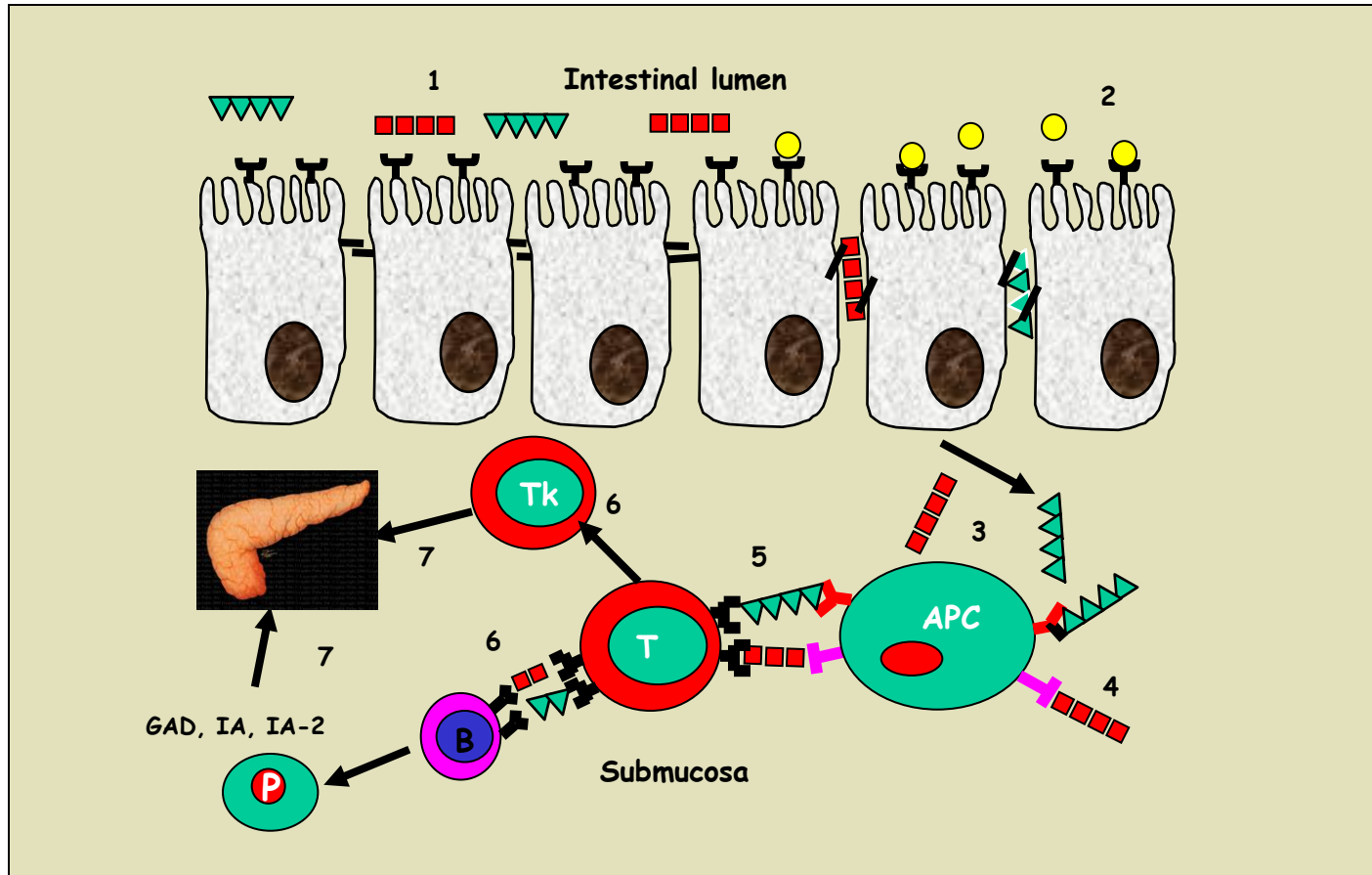
BB/Wor rats that did not develop T1D



Conclusions

- The blockage of the zonulin pathway in BB/wor rats at their preclinical autoimmune stage significantly reduced the progression to T1D up to age 205 days (150 days post-treatment).
- This decreased incidence of T1D was associated to a significant reduction of the anti-GAD antibodies following AT1001 treatment.
- Withdraw of AT1001 treatment was followed by onset of T1D in 33% of the animals.

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 Langerhans islets with subsequent insulin deficiency typical of type 1 diabetes (7).