

**JUVENILE DIABETES: EXAMINING THE PERSONAL  
TOLL ON FAMILIES, FINANCIAL COSTS TO  
THE FEDERAL HEALTH CARE SYSTEM, AND  
RESEARCH PROGRESS TOWARD A CURE**

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**HEARING**

BEFORE THE

COMMITTEE ON  
GOVERNMENTAL AFFAIRS  
UNITED STATES SENATE  
ONE HUNDRED EIGHTH CONGRESS

FIRST SESSION

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JUNE 24, 2003  
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Printed for the use of the Committee on Governmental Affairs



U.S. GOVERNMENT PRINTING OFFICE

88-931 PDF

WASHINGTON : 2003

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**FOR THE  
COMMITTEE ON GOVERNMENTAL AFFAIRS**

**UNITED STATES SENATE**

**June 24, 2003**

Dear Chairwoman Collins, and Members of the Committee:

Thank you for holding the hearing on “Juvenile Diabetes: Examining the Personal Toll on Families, Financial Costs to the Federal Health Care System, and Research Progress Toward a Cure”. We appreciate the opportunity to present our statement for the record.

Type I Diabetes is among the most frequent autoimmune disorders in the pediatric population affecting 1 out of 360 children. The trigger of the autoimmune destruction of the pancreatic cells producing insulin is still unclear mainly because the environmental triggers that induce this damage are unknown. Conversely, the environmental trigger (gluten containing grains) that causes celiac disease, another autoimmune disorder that affects as many as 1 out of 133 children and adults worldwide, is well known. Therefore, an effective treatment is available.

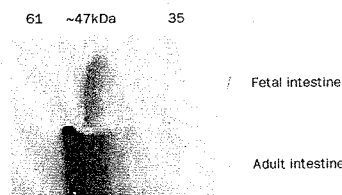
Type I Diabetic children are at a much higher risk to have celiac disease as compared to the general population. There is now growing evidence that this coexistence is not merely due to common genetic predisposition, rather untreated celiac disease can lead to Type I Diabetes.

Therefore, an increased awareness of celiac disease and an aggressive screening campaign to identify celiac disease patients at their early clinical stage will represent an unparalleled strategy to prevent Type I Diabetes in a subset of children that will develop this devastating condition. It should also be stressed that the lifestyle of children affected by both Type I Diabetes and Celiac Disease is much more complicated than children affected by either one of these conditions. Glucose control, the treatment costs, the clinical and management challenges are definitely much more complicated in children suffering from both conditions.

Recent studies conducted at the Center for Celiac Research (CFCR) at the University of Maryland shed some light on the rationale for this coexistence. It is now well established that it is in the interplay between environmental factors and specific susceptibility genes that dictate the immune response for the onset of autoimmune diseases. This interplay can only occur if the intestinal barrier that represents the limiting step for passage of environmental triggers into the body is jeopardized. Zonulin, a molecule recently discovered at the CFCR is the gatekeeper that controls this passage. It is now clear that both Celiac Disease and Type I Diabetes are characterized by a leaky gut as the consequence of an aberrant production of zonulin. This information will be instrumental to develop new preventive and treatment strategies to tackle these devastating conditions.

Thank you for giving us the opportunity to provide this statement.

ATTACHMENT—Lancet. 2000 Apr 29;355(9214):1518-9. Zonulin, a newly discovered modulator of intestinal permeability, and its expression in coeliac disease. Fasano A, Not T, Wang W, Uzzau S, Berti I, Tommasini A, Goldblum SE.



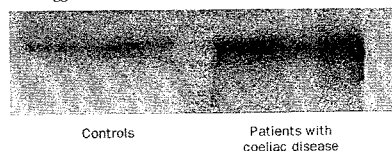
**Figure 1: Immunoblotting of human intestinal tissues with affinity-purified polyclonal anti-ZOT antibodies**

Proteins in tissue lysates of human fetal and adult intestine were subjected to sequential purification steps, resolved by sodium dodecylsulphate polyacrylamide gel electrophoresis, transferred onto polyvinylidene difluoride membranes, and probed with affinity-purified anti-ZOT antibodies. A single protein was purified that migrated with an apparent relative molecular mass of about 47 kDa and immunoreacted with anti-ZOT antibodies.

screen for one or more human intestinal ZOT analogues. Non-primate intestinal tissues were used as an indicator system to identify and purify this analogue. Fetal and adult tissues were obtained from the brain and tissue bank for developmental disorders at the University of Maryland. A single protein (that we named zonulin) with a molecular weight of about 47 kDa was purified to homogeneity from both adult and fetal intestine (figure 1). To establish whether zonulin preparation was biologically active, it was tested on Rhesus monkey intestine with an *ex vivo* assay.<sup>1</sup> Intestinal tissues from the same animal with similar baseline tissue resistances were simultaneously exposed to either zonulin or media alone. Zonulin reversibly increased the monkey intestinal permeability compared with the media control in both jejunum (mean 35.0 [SE 1.8]% vs 3.0 [1.5]% permeability increment;  $p < 0.0001$ ) and ileum (26.0 [5.6] vs 4.9 [1.5] permeability increment), but not in the colon (1.3 [0.6] vs 1.1 [0.5] permeability increment,  $p = 0.37$ , Student's *t* test). This increased permeability allowed the transepithelial passage of insulin, a macromolecule normally not absorbed when given orally.<sup>2</sup>

To establish whether zonulin is perturbed during coeliac disease, a condition in which tight junctions are opened through an as yet undefined mechanism,<sup>1</sup> intestinal tissues were obtained from seven patients with active coeliac disease and six controls and probed for zonulin with anti-ZOT antibodies. Immunofluorescence analysis of coeliac disease tissues showed enhanced zonulin expression within the intestinal submucosa with a characteristic reticular pattern that was consistently absent in control tissues. Quantitative immunoblotting of intestinal tissue lysates from patients with active coeliac disease confirmed higher zonulin protein concentrations than in control tissues (figure 2).

Since intestinal zonulin expression was increased during the acute phase of coeliac disease, when tight junctions are opened, this suggests a causal role of this endogenous mediator in



**Figure 2: Zonulin protein in intestinal tissues from coeliac disease patients and controls**

The increased expression of zonulin in intestinal tissues from coeliac patients was confirmed by western analysis. The amount of zonulin normalised to the total protein content in the tissues analysed was about 3-fold higher in intestinal specimens from patients with coeliac disease than in control tissues. These blots are representative of six specimens.

### Zonulin, a newly discovered modulator of intestinal permeability, and its expression in coeliac disease

Alessio Fasano, Tarcisio Not, Wenle Wang, Sergio Uzzau, Irene Berti, Alberto Tommasini, Simeon E Goldblum

We identified zonulin, a novel human protein analogue to the *Vibrio cholerae* derived Zonula occludens toxin, which induces tight junction disassembly and a subsequent increase in intestinal permeability in non-human primate intestinal epithelia. Zonulin expression was raised in intestinal tissues during the acute phase of coeliac disease, a clinical condition in which tight junctions are opened and permeability is increased.

We have shown that zonula occludens toxin (ZOT), a protein elaborated by *Vibrio cholerae*, reversibly regulates the permeability of tight junctions.<sup>1</sup> ZOT interacts with a specific surface receptor<sup>1</sup> with subsequent protein kinase C  $\alpha$ -dependent polymerisation of actin microfilaments strategically localised to regulate the paracellular pathway. On the basis of this observation, we investigated whether ZOT might mimic an endogenous modulator of tight junctions. We also postulated that ZOT and its putative eukaryotic analogue could be structurally and immunologically related.

Accordingly, specific anti-ZOT antibodies and an *ex vivo* intestinal permeability assay<sup>1</sup> were used in combination to

coeliac disease pathogenesis. Further, this increased expression of zonulin in the face of tight junctions disassembly might allow zonulin presentation to the submucosal gut immune system. Accordingly, we used a ZOT-based ELISA to detect antibodies to zonulin in the serum samples of patients with coeliac disease and controls. Anti-zonulin IgG was not higher in patients with coeliac disease than controls. By contrast, anti-zonulin IgA was raised in the serum samples of 25 of 117 (21%) patients with coeliac disease during the acute phase of the disease but in none of the 30 patients in remission ( $p < 0.0001$ ). Only nine of 163 (6%) healthy controls had a minimally but significantly increased anti-zonulin IgA titre ( $p < 0.0001$ ). The incidence of anti-zonulin antibodies during the acute phase of coeliac disease is consistent with the incidence of other auto-antibodies described in coeliac disease.<sup>4</sup> In seven patients with coeliac disease followed longitudinally, the raised anti-zonulin IgA returned to normal after 3–6 months symptomless remission on a gluten-free diet.

It has been recently reported that untreated coeliac disease predisposes to autoimmune disorders such as insulin-dependent diabetes mellitus, Hashimoto's thyroiditis, autoimmune hepatitis, and connective tissue diseases.<sup>4</sup> Perhaps zonulin opens small intestinal tight junctions during the early stage of coeliac disease and allows entry of putative allergens into the intestinal submucosa, in which an autoimmune response is elicited. In a spontaneous diabetic rat model,  $\beta$ -islet cell destruction and other autoimmune features develop only 3–4 weeks after the rise in gastrointestinal paracellular permeability.<sup>5</sup> Notably, these permeability changes always precede the autoimmune process.<sup>5</sup> Further, the barrier dysfunction is restricted to the small intestine,<sup>6</sup> paralleling the regional distribution of the zonulin regulatory system within the gastrointestinal tract.<sup>1</sup> Our findings that enhanced intestinal permeability in this diabetic rat model was associated with increased concentration of intraluminal zonulin (unpublished) further supports the pathogenic role for this protein at the onset of autoimmune disorders, such as diabetes mellitus and coeliac disease.

Our results support the idea that zonulin participates in the physiological regulation of intercellular tight junctions in the small intestine. Dysregulation of this conceptual zonulin model might contribute to the perturbation of the intestinal barrier functions, leading to the passage of environmental antigens involved in the pathogenesis of coeliac disease and related autoimmune disorders.

A F was partly supported by the National Institute of Health grant DK-48373.

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